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COMMENTARY

Oral Complications of Cancer Chemotherapy: Present Status and Future Studies[1]

Douglas E. Peterson[2,*] and Stephen T. Sonis[3,4]

Chemotherapy has led to improvements in survival for leukemia, lymphoma, testicular cancer, small cell lung cancer, and other neoplasms. Myelosuppression is often associated with this treatment; the more profound and prolonged the myelosuppression, the more serious the complications that may develop. Management of these complications is critical for short-term treatment, patient comfort, and ultimately patient survival.

The oral cavity is a site in which complications secondary to cancer treatments frequently develop (1–7). However, current diagnosis and management of these sequelae within the oral cavity are relatively limited in scope. Variables such as disease type, treatment program orientation, health professional motivation, and recognition of the potential problems in part dictate the approach to these complications. Often the clinical situation in which oral pathology is not accurately diagnosed and treatment becomes inadequate leads to even more serious sequelae.

A recent symposium[5] representing medicine, dentistry, nursing, dental hygiene, and pharmacy identified several areas of chemotherapy-associated oral complications, both infectious (mucositis, periodontal infection, and dental pulp infection) as well as noninfectious (xerostomia, anemia, neurotoxicity, and thrombocytopenia). The purpose of this report is to review these sequelae, as well as avenues for their future research. This review should identify the need for the dental staff to play an integral role in the prevention, recognition, and treatment of oral sequelae. By incorporating such a staff as part of the overall oncologic team, the supportive care needs of the patient receiving chemotherapy will be better met.

INFECTIOUS COMPLICATIONS

Complications of the oral cavity are frequently seen following the administration of many cancer chemotherapies. Approximately 40% of all patients receiving drug therapy develop oral problems as a direct consequence of treatment (1). Oral complications may be caused by the nonspecific action of the chemotherapeutic agent on the rapidly dividing cells of the basal epithelium, resulting in ulceration and destruction of the mucosa (mucositis). Alternatively, chemotherapy-induced myelosuppression may exacerbate chronic oral infections such as periodontal or pulpal disease, produce local secondary infection of ulcerated mucosal tissue, or promote oral bleeding. Clearly, the loss of an intact epithelial barrier in a site which tends to be heavily colonized by potential bacterial pathogens may have serious consequences for the patient.

Mucositis (fig 1)

The development of mucositis in myelosuppressed patients is varied. Some chemotherapeutic agents are associated with a high incidence of oral mucositis (eg, methotrexate or doxorubicin), but not all patients receiving these agents develop even mild mucositis. The pathogenesis of mucositis is different from that of acute periodontal infection, which arises in a site of previous chronic infection; chemotherapy-induced oral mucositis arises on a usually intact mucosa. The drugs are directly toxic to cells which are undergoing rapid replacement (8–11); once the break in mucosal integrity occurs, secondary infection can readily occur. In addition, mucositis may

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[5]Oral Complications of Cancer Chemotherapy, Baltimore, MD, Sept 21–22, 1981.

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FIGURE 1.—Oral mucositis of the palate secondary to chemotherapy (arrow). This lesion appeared approximately 1 wk after initiation of therapy on tissues which were clinically normal prior to the treatment (photo courtesy of Ms. Lisa T. Williams, RDH).

impair the patient's nutritional intake because of the discomfort of mastication and the burning sensation of some mildly acidic foods. Microbial cultures and appropriate antibiotic therapy should be considered for all such lesions that are acutely infected.

The treatment of oral mucositis in general remains palliative and symptom-oriented. There appear to be few ongoing prospective studies to determine susceptibility to the development of such a lesion, or ideally, the means by which the lesion might be prevented from initially developing. Oral hypothermia techniques are being evaluated in some centers; questions as to the effect of such procedures on antitumor treatment as well as lack of delivery to the remainder of the gastrointestinal mucosa persist. Although some studies have suggested that good oral hygiene reduces oral mucositis (12–14), this has not been our experience. Successful management ideally should include elimination of the toxic effects on the mucosa and prevention of the mucosal ulceration wherever possible. This prevention might include measures directed against oral microbial flora, since it has been shown that germ-free animals have mucosal cell renewal rates that are one half those of conventional animals (15).

Periodontal Infection (fig 2)

Periodontal complications occur more frequently than is generally realized. Periodontal disease is extremely prevalent, affecting up to 98% of the population of this country (16). This prevalence is also found in patients scheduled to receive myelosuppressive chemotherapy (table 1). While recent reports have described the impact of oral care protocols on infection in myelosuppressed patients (12–14,17–19), many oncologists appear reluctant to institute such care for fear of causing bacteremias in their patients. One should note that transient bacteremias can occur during mastication (20), in the presence or absence of clinical gingival disease (21,22). The prevalence of periodontal infection, its management, and its possible prevention in myelosuppressed patients with acute nonlymphocytic leukemia (ANLL) have been documented (6,7). Approximately 28% of all acute infections which developed during remission induction represented acute exacerbations of pre-existent periodontal disease. In most cases, the inflammation associated with this lesion was markedly suppressed, similar to the lack of inflammation at other sites in this clinical setting (23,24). Thus, the diagnosis is more difficult to make, and one must have a high index of suspicion. Intensive oral hygiene before and during myelosuppressive therapy has proven efficacious in preventing many of these infections in patients with ANLL (6). This prophylactic therapy does not significantly increase other adverse clinical sequelae, such as esophageal, pharyngeal, or pulmonary infections. In patients with gingival infiltrate and engorgement

FIGURE 2.—Acute periodontal infection (arrow) in a granulocytopenic (< 100 cells/μl) patient with acute leukemia. Unlike oral mucositis, this lesion represents an acute exacerbation of a previously chronically infected site (photo courtesy of Ms. Lisa T. Williams, RDH).

secondary to acute monocytic or myelocytic leukemia or histiocytosis X, good oral hygiene can be extremely difficult to achieve. Such oral care does not appear to reduce acute periodontal infection in patients with leukemic infiltrates; further study as to effective oral care for this group of individuals is needed.

Since periodontal complications are infectious in nature, the clinician must be cognizant of changes in the oral microbial flora during treatment. Forty-seven percent of all acute infections in the myelosuppressed leukemic patient have been shown to be caused by organisms acquired in the hospital (25). It is also known that there is a shift in the oral microbial flora to enteric organisms as a consequence of disease and debilitation (26). Shifts similarly occur in the population of microbial flora within the oral cavity of patients with acute leukemia, with enteric gram-negative bacteria becoming predominant (27,28).

TABLE 1.—Status of periodontium at admission in patients with ANLL

Periodontal status	No. of patients
Healthy	0
Gingivitis only	2
Moderate disease	29
Severe disease	35
Total	66

It has been shown among immunodeficient patients that the supragingival plaque has higher levels of catalase-negative diphtheroids and *Candida sp.* than among normal individuals (29). There are, however, virtually no data available regarding the subgingival periodontal flora in patients receiving chemotherapy. It can be assumed that, similar to immunodeficient patients, there are probably profound changes in patients debilitated by or receiving myelosuppressive therapy. These changes are of major significance in treating both periodontal and mucosal infections in the myelosuppressed patient. Surveillance cultures are often used to monitor oral microbial changes in such patients (30). However, these techniques do not always directly sample flora contiguous to the ulcerative lesion of periodontal disease. Thus, a study (*a*) assessing shifts in the periodontal flora during myelosuppressive chemotherapy and (*b*) identifying etiologic agents causing acute periodontal infections has been instituted at the University of Maryland Cancer Center. To date, preliminary data from 20 patients suggest that the acute periodontal exacerbations are not caused by major pathogens associated with mortality in ANLL [eg, *Klebsiella sp.*, *Proteus sp.*, *Escherichia coli*, *Aspergillus sp.*, and *Pseudomonas sp.* (25,31)]. In addition, it appears that there is a quantitative but not qualitative shift in the microbial population during profound, prolonged granulocytopenia. Further study is needed to confirm and elaborate these find-

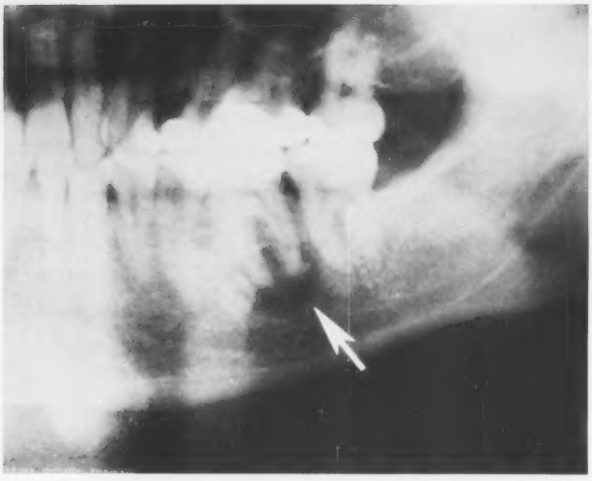

FIGURE 3.—Roentgenograph of periapical pathosis (arrow) of mandibular left first molar. This patient was granulocytopenic at admission, was febrile (38.3°C), and complained of intense pain in this region. This acute infection should be treated by either tooth extraction (if appropriate) or endodontic therapy (root canal).

ings. The techniques utilized in the analysis of such organisms are tedious and specialized; the periodontal flora are usually gram-negative anaerobes resident within the periodontal pocket (32,33) and are fastidious in their growth requirements. The laboratory must be equipped to culture these microbial agents; otherwise, accurate identification is not likely.

Dental Pulp Infections (fig 3)

Chronic infections of the dental pulp may also become a source of significant systemic infection during periods of myelosuppression. A thorough dental examination, including roentgenographs, is frequently the only way such pathology can be identified. Between 5% and 10% of cancer patients demonstrate roentgenographic evidence of pulpal infection of sufficient magnitude to cause bony involvement. The optimal treatment of involved teeth during granulocytopenia remains unresolved. However, elimination of the source of the infection, either by tooth extraction or by pulpal extrication, may often be the treatment of choice.

While these issues require further study, it is clear that tooth extractions in patients with profound, prolonged myelosuppression can be associated with serious systemic complications. However, a recent study has shown that by adhering to stringent guidelines the potential for such complications is minimized (34). Briefly, these guidelines include (*a*) antibiotic prophylaxis if the patient's granulocyte count is $< 2000/\mu l$, (*b*) use of effective platelet transfusions (ie, those which produce increments including matched platelets where needed) if platelet count is $< 40,000/\mu l$, (*c*) avoidance of intra-alveolar hemostatic packing agents, (*d*) primary closure and alveolectomy at time of surgery, and (*e*) if possible, waiting at least 10 days between extraction date and induction of granulocytopenia ($< 1000/\mu l$) secondary to chemotherapy.

NONINFECTIOUS COMPLICATIONS

Noninfectious complications that can arise in patients receiving cancer treatment include xerostomia, anemia, neurotoxicity, and thrombocytopenia.

Xerostomia is a transient development secondary to some chemotherapeutic agents; the patient complains of a generalized dryness of the oral cavity. The lesion can be confirmed by milking of the parotid and submandibular glands and noting decreased quantity of salivary flow. In addition, the oral mucosal membranes will often appear atrophic and dry. The patient should be informed as to the temporary nature of the condition. Palliative relief can be obtained by the use of Xero-Lube (Scherer Laboratories, Dallas, TX), a glycerine-based solu-

tion containing sodium fluoride, or oral rinses with 0.1% aqueous sodium carboxymethylcellulose (35). Since a xerostomic condition may predispose to oral mucositis, it probably merits palliative topical therapy.

The most striking oral findings with anemia may be atrophic tongue and buccal mucosa, as well as generalized pallor. Obviously, this condition will persist until normal marrow function resumes. The direct effects of the chemotherapeutic agents may also predispose the patient to a neuropathy, with symptoms in the oral cavity that may mimic those of odontogenic or periodontal origin. In this clinical setting, the clinician should look for obvious oral disease, such as dental pulpal pathology or acute periodontal infection; if there is none, no active dental therapy is recommended, since the condition is usually reversible.

Thrombocytopenia per se rarely results in profound oral complications. Oral bleeding is a relatively common consequence and may occur in one of several forms. Gingival bleeding is seen with platelet counts $< 15{,}000/\mu l$ and is generally more severe in patients with pre-existing periodontal disease or poor oral hygiene. Hematoma formation as a result of trauma is often exaggerated when platelet counts decrease significantly below $50{,}000/\mu l$. Secondary infection of areas of hematoma are also a concern in the myelosuppressed patient. Finally, extravasation of blood into mucosal tissues, especially sublingually, may occur, but respiratory embarrassment is a rare sequela.

To control minor local oral bleeding, topical thrombin (100–100,000 units/ml) can be used. It should be remembered that, even in a thrombocytopenic patient, gingival bleeding can indicate inflammation. Failure to maintain excellent oral hygiene can result in increased oozing compared to that seen with the proper use of oral hygiene instruments, such as toothbrush and dental floss. Appropriate platelet transfusions (random donor platelets for most and matched platelets for immunized patients) can usually stop most of this type of bleeding.

FUTURE DIRECTIONS

The major goals of oral care in the cancer patient receiving chemotherapy are: assessment and treatment prior to chemotherapy, maintenance of good oral hygiene, patient education, and diagnosis and treatment of oral complications. Dental services should be part of the routine care of the patient with cancer. In many cases, the size and scope of the facility will dictate the extent to which this care can be provided. The dental staff should be contacted and those with interest and knowledge in the field should be recruited to evaluate and administer oral treatment to patients receiving chemotherapy. In institutions with teaching services, dental residents and attending staff should be afforded facilities necessary to provide comprehensive diagnosis and treatment of odontogenic pathology and management of soft tissue complications. If no hospital-based dental program exists, the patients could possibly be referred to their own dentists for evaluation; however, the oncologist should clearly specify to the dentist the nature of the concerns.

The future of supportive oral care for the patient receiving chemotherapy lies in a comprehensive cooperative team approach. To improve the ability of the dental team in providing basic supportive services, there are four areas that require further investigation.

1. Almost all clinical decisions regarding oral complications are currently based on limited data. There is a major need for prospective, well-controlled clinical studies on the subject. These studies should include analysis of (*a*) complications of dentulous and edentulous patients, (*b*) topical local therapy to reverse or prevent complications arising from systemic therapy, (*c*) new approaches to local control of infection and bleeding resulting from myelosuppression, (*d*) long-lasting topical palliative rinses, and (*e*) effect of oral hygiene on complications of chemotherapy.

2. There is a need for a classification system for oral complications of chemotherapy, since currently there are no standards for collection and analysis of such data. As a result, data cannot easily be compared among institutions. It is conceivable that a national registry for reporting, documentation, and analysis of oral complications of chemotherapy may be developed to allow data sharing.

3. Animal models need to be established to evaluate variables affecting the frequency and severity of oral complications as well as methods for therapeutic intervention.

4. Education of health care professionals concerning the need for a multimodality approach to the prevention and treatment of oral complications is essential.

The field of oral complications of cancer chemotherapy has had a comparatively limited development. Further research in this area will provide for better patient care.

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Chemotherapy Sensitivity Assessment of Leukemic Colony-Forming Cells With In Vitro Simultaneous Exposure to Multiple Drugs: Clinical Correlations in Acute Nonlymphocytic Leukemia[1,2]

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SUMMARY

For 13 patients with acute nonlymphocytic leukemia receiving four-drug combination chemotherapy (14 chemotherapy trials), leukemic bone marrow cells were obtained before treatment and were exposed in vitro to all four-drug-mixture solutions simultaneously followed by the survival assessment of leukemic colony-forming cells (L-CFU). Survival of normal marrow colony-forming cells (CFU-C) was also assessed concurrently, and the sensitivity index (SI) of leukemic cells was determined as the ratio of the survival of CFU-C to that of L-CFU. Correlations of in vitro results to clinical results were excellent: four of five trials resulting in complete remission had high SI and eight of nine trials without complete remission had low SI ($P = 0.02$). The potential advantages of this method of exposing cells to drug mixture are discussed in terms of detecting drug synergism and improving the efficiency of in vitro chemotherapy sensitivity studies.

[Cancer Treat Rep 66:1257–1261, 1982]

Our recent studies indicate that in vitro chemotherapy sensitivity of leukemic colony-forming cells (L-CFU) correlates well with clinical response to the same chemotherapy in individual patients with acute nonlymphocytic leukemia (ANLL) (1,2). All nine patients reported in our last study received combinations of two to four chemotherapeutic drugs, while in vitro chemotherapy sensitivity was assessed with component single drugs separately (2). Synergistic effects of component single drugs appear to be important in combination chemotherapy, and this synergistic effect is not reflected in vitro unless cells are exposed to the drugs together. The most straightforward initial step in this direction involves the use of mixture solutions of all the component single drugs. There are added advantages of using a drug mixture. Testing only one solution instead of multiple solutions of single drugs reduces the time, effort, expense, and number of cells required. Therefore, the logical extension of our last study with single drugs was to investigate multiple-drug sensitivity of L-CFU.

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MATERIALS AND METHODS

Patients

Patients with newly diagnosed ANLL, without prior treatment, were candidates for this study. They

TABLE 1.—Clinical and in vitro chemosensitivity data of 13 leukemic patients

Patient/case No.	Age (yrs)	Sex	Diagnosis*	Treatment	Complete remission†	SI‡			
						Drug-mixture		Single drugs	
						SI-Mxa	SI-Mxb	SI-Cy	SI-An
1	69	M	AMML	AdOAP	Yes	2.91	3.49	0.97	3.0
2	51	F	AMML	ROAP	Yes	1.74	2.47	2.82	3.35
3	56	F	AMML	ROAP	Yes	1.89		1.5	1.2
4	45	F	AML	ROAP	Yes	5.92	6.73	0.64	0.88
5§	44	F	AML	AdOAP	Yes	0.69	0.87		
6	56	M	AMML	ROAP	No	0.57		1.03	0.96
7¶	59	M	AML	AdOAP	No	0.95		1.04	0.68
				ROAP	No	0.87		1.04	1.23
8	57	F	BT-CML	ROAP	No	0.95	0.91		
9	19	M	AMML	ROAP	No	0.61	0.74		
10	55	F	AML	ROAP	No	0.95	0.95		
11	69	F	AMML	AdOAP	No	0.79	0.81	0.72	0.63
12**	51	M	AML	ROAP	No	0.79	1.15	0.83	0.95
13††	62	M	AMML	ROAP	No	1.27	0.92	1.18	1.31

*AMML = acute myelomonocytic leukemia, AML = acute myelocytic leukemia, and BT-CML = blast transformation of chronic myelocytic leukemia.

†Complete disappearance of all evidence of disease, with normal marrow (blast cells < 5%).

‡In vitro chemotherapy sensitivity index (SI) calculated on the basis of either 1 concentration (SI-Mxa) or 4 concentrations (SI-Mxb) of drug mixtures and single drugs, cytarabine (SI-Cy) and anthracycline (SI-An) (*see* Materials and Methods).

§Good complete remission achieved with a long survival (23+ mos). However, the plating efficiency was low (12.4 ± 1.5 colonies/ 5×10^5 cells plated).

¶Patient failed therapy both with AdOAP and later with ROAP.

**Partial remission achieved with fairly good survival (13+ mos).

††Patient died in 3 mos with no evidence of response.

were given intensive combination chemotherapy, consisting of vincristine, cytarabine, and prednisone with either doxorubicin (AdOAP) (3) or zorubicin (ROAP) (2), to induce complete remission. Bone marrow was aspirated before chemotherapy for routine morphologic evaluation and in vitro chemotherapy study (see below). The subjects of this analysis are the initial 13 patients for whom both clinical response is evaluable and in vitro data are available (table 1). Complete remission in five patients from a sample of 13 patients is statistically acceptable assuming a true response rate of 60% for the population ($P = 0.10$ on the basis of binormal distribution). Normal bone marrow for control culture was obtained from hematologically normal patients with solid tumors undergoing bone marrow examination as part of the pretreatment staging workup. Informed consent was obtained prior to marrow aspiration from all patients in accordance with guidelines established by the University of Kansas Human Subjects Committee.

Cell Culture Assay

A detailed description of the cell culture method used in this study has been reported previously (4). Briefly, the culture system consisted of two layers of 0.3% agar in a 35-mm plastic Petri dish perforated at the bottom by five small holes. The top agar layer contained twice-washed buffy coat cells separated from bone marrow aspirates. Both layers contained Alpha medium (GIBCO, Grand Island, NY) with 15% fetal calf serum (Flow Laboratories, Rockville, MD) and 15% leukocyte-conditioned medium. The latter was prepared by incubation of normal human peripheral leukocytes with phytohemagglutinin (Wellcome Research Laboratories, Beckenham, England). Cultures were incubated for 2–3 weeks at 37°C in an atmosphere continuously flushed with 7% CO_2. Daily throughout the incubation period, each culture dish was removed from the incubator and fed from the top with 0.5 ml of Alpha medium containing 15% each of fetal calf serum and leukocyte-conditioned medium. The fed medium diffused through the agar layers and drained out of the dish through the holes on the bottom. Colonies of ≥ 50 cells were counted using an inverted microscope. Quadruplicate dishes were routinely set up for each experimental group of cultures, and the mean numbers of colonies were used to calculate chemotherapy sensitivity indices (see below). Plating efficiency varied considerably among different patients, and the cultures were set up initially with 5×10^5 cells (maximum number of cells which could rea-

sonably be plated in a culture dish) to determine the plating efficiency for individual patients. If the number of colonies was less than ten at this cell concentration, chemotherapy studies were not performed in that patient; five of 18 patients (28%) could not be studied for this reason. On the other hand, in patients with high plating efficiency the number of plated cells was appropriately reduced to yield between 50 and 300 colonies. The number of colonies obtained for 13 patients in this study ranged between 12.4 and 266 (median = 78) for between 2×10^4 and 5×10^5 (median = 2×10^5) cells plated. Bone marrow cells were routinely stored in liquid nitrogen, and all the culture studies were done on these frozen cells as described previously (2,5). Previous studies (2,6,7) have substantiated that colonies growing in this culture system from leukemic bone marrow are leukemic in origin (L-CFU), while normal marrow gives rise to normal myeloid colonies (CFU-C).

In Vitro Chemotherapy Sensitivity Determinations

Vincristine, cytarabine, and doxorubicin were obtained in pure powder form without preservatives from respective manufacturers (Lilly, Indianapolis, IN; Upjohn, Kalamazoo, MI; and Adria, Columbus, OH). Clinical preparations were used for zorubicin and prednisolone (a hydroxylated compound active also in vitro) obtained from the National Cancer Institute, Bethesda, MD. A 1-hour pulse exposure of cells to drugs was done as in the previous study (2) except that a four-drug-mixture solution was used in addition to single-drug solutions. The four-drug solutions contained either doxorubicin or zorubicin in addition to vincristine, cytarabine, and prednisolone depending on whether the patient received either AdOAP or ROAP. The concentration of drugs that singly reduced the average survival of CFU-C to 75% (1,2) was used as the basis in making a standard drug-mixture solution. It was reasoned that this concentration of drug would be most effective in detecting differences in effect on survival of CFU-C and L-CFU, when L-CFU is more sensitive than CFU-C. If lower concentrations are used, lethal effects of L-CFU might not be sufficiently detected while greater concentrations of drug would tend to diminish differences in survival between CFU-C and L-CFU (2). For vincristine and prednisolone, one fifth of these indicated concentrations was arbitrarily used, because these two drugs probably play a minor role in the induction of complete remission, and the indicated concentrations appear too high in view of pharmacokinetic data (8).[6] Individual drug solutions for these four drugs were prepared and stored separately and were combined in equal volumes immediately prior to experiments to make drug-mixture solutions. Thus, the standard drug-mixture solution contained 5×10^{-8} M vincristine, 1×10^{-5} M cytarabine, 1×10^{-4} M prednisolone, and either 1×10^{-7} M doxorubicin or 1×10^{-7} zorubicin. Depending on their availability, bone marrow cells from each patient were tested either with this standard drug mixture only or also with five, ten, and 20 times this drug concentration. Whenever a leukemic bone marrow was tested for L-CFU survival, a normal bone marrow was also tested simultaneously and in identical fashion for CFU-C survival. The average survival rates of CFU-C with one-, five-, ten-, and 20-fold drug-mixture concentrations were 70%, 40%, 20%, and 3%, respectively, with an approximate coefficient of variation of 20%–30%. Because of this day-to-day variation, simultaneous CFU-C control is needed, as fully discussed and substantiated previously (2), to obtain stable data for chemotherapy sensitivity of L-CFU, which can be expressed in relation to that of CFU-C as follows:

$$\text{Sensitivity index (SI)} = \frac{\%\ \text{survival of CFU-C}}{\%\ \text{survival of L-CFU}}.^{7}$$

For SI calculated on the basis of one-time drug mixture only (SI-Mxa), simply the ratios of survival fractions of CFU-C and L-CFU were taken. If the data from four different drug-mixture concentrations were used, the areas under the survival curves were calculated in the manner described (9) for CFU-C and L-CFU and the ratios of these two areas were taken to yield SI-Mxb. SI for single drugs, either for cytarabine (SI-Cy) or anthracycline (SI-An), were based on one drug concentration (2). As can easily be seen from the formula, SI values > 1 indicate that L-CFU are more sensitive than CFU-C, and $SI < 1$ indicate that L-CFU are more resistant than CFU-C.

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[6]Townsend RJ (Medical Bioavailability Unit, Upjohn Co, Kalamazoo, MI). Personal communication regarding prednisone-prednisolone pharmacokinetic data, 1981.

[7]The survival fractions of CFU-C and L-CFU can fluctuate from one experiment to another. However, this fluctuation tends to occur in the same direction and by a comparable magnitude for a given set of experiments. Therefore, the ratio of these two fractions (ie, the SI) remains relatively constant. This was systematically studied and proven in the previous study (2). One of the examples in case 3 with zorubicin is shown as follows: SI 1.20 (49%/41%), SI 1.21 (80%/66%).

RESULTS

Table 1 shows in vitro chemotherapy sensitivity of L-CFU along with clinical data on 13 leukemic patients in this study. There were 14 chemotherapy trials, however, because one patient (No. 7) had two trials. For ten patients, enough cells were available to test all four concentrations of drug mixture, and both SI-Mxa and SI-Mxb were calculated. For the rest, only one concentration was tested for SI-Mxa. Therefore, SI-Mxa were available in all trials while SI-Mxb were not. SI for single drugs (SI-Cy and SI-An) were also shown for comparison where available. All SI for single drugs except for patients 4, 12, and 13 have been published previously (2).

Analyzing on the basis of SI-Mxa, four of five trials resulting in complete remission were associated with high indices with only one false low (case 5), and eight of nine trials not resulting in complete remission carried low indices with one false high (case 13). This correlation is statistically significant ($P = 0.02$ by Fisher's exact test), with an overall accuracy of 86% (12 of 14 trials). There was no significant difference between SI-Mxa and SI-Mxb ($P > 0.1$ by paired t test) in terms not only of whether the index was > 1 or < 1 but also of the absolute magnitude of the values. There were only two instances where obvious discrepancies were noted (cases 12 and 13). Because these two discrepancies were in opposite directions of each other, the overall clinical correlation would be the same, even if SI-Mxb were used where available. Data on three cases in which false correlations were noted either by SI-Mxa or SI-Mxb were examined more closely. Patient 5 had a plating efficiency that was quite low, the lowest among all, and therefore the SI based on this plating efficiency is likely to be subject to statistical variations. Patient 12 showed considerable clinical benefit from the chemotherapy, and therefore the high SI (SI-Mxb) might be closer to true sensitivity than the low SI (SI-Mxa). No ready explanation can be found for case 13. SI based on single drugs are generally in fair agreement with SI-Mx. Among five cases where SI-Mxa were > 1, both SI-Cy and SI-An were also > 1 in three cases, and the average value of SI-Cy and SI-An was clearly > 1 in one case (No. 1). Case 4 was exceptional, with exceedingly high SI-Mx in spite of low SI-Cy and SI-An. In five situations where SI-Mxa were < 1, both SI-Cy and SI-An were < 1 (two cases), and the average of these two values was < 1 (two cases) or very close to 1 (case 7, ROAP).

DISCUSSION

Chemotherapy sensitivity assessment on the basis of in vitro colony formation appears to be a reliable indicator for clinical response to the same chemotherapy in human malignant diseases as shown by us for ANLL (2) and by others for a variety of solid tumors (9,10). This can be valuable not only in the selection of the best possible chemotherapy drugs for individual patients but also in the screening of new compounds for further evaluation.

There are, however, a number of complicated factors to be considered. One is that most of the human malignancies, whether leukemias or solid tumors, are currently treated with combination chemotherapy, although most in vitro tests have been done with individual drugs. The synergistic effect between component single drugs appears to be important in combination chemotherapy, and this effect will not be reflected in vitro by exposing cells to single drugs separately. Our study here involves exposing cells in vitro to the mixture of multiple drugs, which may reflect the synergistic effect and thus be a better overall indicator for clinical outcome. The result of this study indeed shows an excellent clinical correlation, thus establishing the feasibility of in vitro multiple-drug exposure. It is, however, difficult to determine to what extent the drug-mixture exposure reveals synergistic effect of component single drugs. There are a limited number of cases where single-drug and drug-mixture exposures can be compared directly, and the results of these two exposures are generally in fair agreement with each other. However, there is a patient (No. 4) with good clinical response where SI-Mx is very high in spite of low SI-Cy and SI-An. This suggests the possibility of drug synergism detection by drug-mixture exposure. Also, in this approach where there is only one solution of multiple drugs to be tested rather than multiple solutions of single drugs, the number of cultures to be set up and the requirement of bone marrow cells are also considerably reduced. This reduces the expense of the study and can be an extremely important practical advantage for patients from whom enough cells are not obtained and/or for whom plating efficiency is low.

We intended to test four different concentrations of drug mixture in all patients. However, enough cells were not available in three patients, so only one concentration, the lowest, was tested. There was fairly good agreement between SI obtained on the basis of one (SI-Mxa) or four (SI-Mxb) concentrations of drug mixture. This also indicates that the values of SI are relatively stable with different drug concentrations. We would still like to test at least one more high concentration in addition to one low concentration, to detect the possible presence of minor cell subpopulations resistant to the drugs. However, if enough cells are not available, a test with one concentration appears to be acceptable.

The drug concentrations used in vitro with satisfactory results in our leukemia studies have been determined by a large number of experiments on CFU-C over many years (1). In more recent solid tumor studies of similar design by others (9,10), choice of drug concentration has been made on the basis of pharmacokinetic studies. It is interesting to note that the concentration of drugs used in both our leukemia system and in solid tumor studies (doxorubicin and vincristine) are remarkably similar. Further, the use of single rather than multiple concentrations of drugs also appears to be satisfactory in the solid tumor studies (10,11).

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Randomized Comparison of 5-FU Alone or Combined With Mitomycin and Cytarabine (MFC) in the Treatment of Advanced Gastric Cancer[1]

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SUMMARY

In a prospective randomized study, 53 patients with advanced gastric cancer previously untreated by chemotherapy were randomly assigned to treatment with 5-FU alone or combined with mitomycin and cytarabine (MFC). The response rate with MFC was higher (28%) than with 5-FU alone (21%). The difference, however, was not statistically significant. Median response duration was practically identical with 5-FU (16 weeks) and MFC (17 weeks). There was no difference in median survival between the two groups (39 and 43 weeks, respectively).

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Combination chemotherapy is usually considered to be more effective in advanced gastric carcinoma than in any other gastrointestinal neoplasm. Combinations of 5-FU and nitrosoureas have been reported to produce objective response rates greater than 5-FU alone (1). Other subsequent phase II studies involving combinations of mitomycin and cytarabine (Ara-C) with 5-FU gave higher response rates than those usually seen with 5-FU alone (2). More recently, a 50% response rate has been reported in gastric carcinoma treated with the combination of doxorubicin, mitomycin, and 5-FU (3). Until now, however, there has been no evidence, based on a prospective randomized comparison, of a statistically significant superiority in the remission rate obtained by a combination of agents in comparison to 5-FU alone.

The purpose of the present prospective randomized study is to compare the efficacy of 5-FU alone to that of the combination of mitomycin, 5-FU, and Ara-C (MFC) in advanced gastric carcinoma.

MATERIALS AND METHODS

Patient Characteristics

Between January 1976 and December 1979, 53 consecutive patients with histologically proven gastric carcinoma were entered in this study. All patients had either advanced disease beyond the possibility of a surgical excision or a recurrence following primary surgical treatment. At least one measurable or evaluable parameter of disease, evidenced either directly or with instrumental examination, was required. Patients with an expected survival < 1 month, with other tumors, or with brain metastases were excluded. Laboratory requirements at the start of treatment were bilirubin < 3 mg/100 ml and creatinine < 1.5 mg/100 ml. Practically all patients were ambulatory. No patient had received previous cytotoxic chemotherapy or radiotherapy.

The 49 evaluable patients showed the following characteristics and distribution between the two treatment arms: median age was 64 years in both arms; 16 males and eight females received 5-FU and 19 males and six females received MFC; Karnofsky performance status was 100–80 in 16 and 17 patients, respectively, 70–50 in seven and eight, and < 50 in one who received 5-FU and in none who received MFC; the primary tumor had been resected in eight and nine patients, respectively, and unresected in 16 patients in each treatment arm; sites of indicator lesions were abdomen in six and five patients, respectively, liver in seven and ten, stomach in 16 and 17, and other sites in five and four. Treatment was assigned by randomization and was performed in our institution. Patients were directly and continuously followed by the same doctor during treatment and, if possible, during all subsequent chemotherapy regimens until death.

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Treatment Procedure

Patients were randomized between two arms of treatment, 5-FU or MFC. They were stratified according to sex, age (< or > 60 years), and status of primary tumor. Drugs were given iv with the following schedules.

Arm A.—5-FU was given at the dosage of 13.5 mg/kg/day by bolus injection for 5 consecutive days, followed by a half-dose every other day until the appearance of toxicity. The treatment was then stopped and promptly resumed after recovery from gastrointestinal and hematologic toxicity. The treatment was then continued indefinitely, with 5-FU given at weekly doses of 15 mg/kg/iv.

Arm B.—MFC was administered twice a week for the first 2 weeks and then once a week indefinitely in the following doses: mitomycin, 0.08 mg/kg/iv; 5-FU, 10 mg/kg/iv; and Ara-C, 0.8 mg/kg/iv.

The dose of 5-FU was reduced if marked gastrointestinal toxicity appeared during the first 5 days of treatment. The following dose attenuation schedule for bone marrow depression was used in both arms: 100% of the dose for patients with wbc count $\geq 4000/mm^3$ and platelet count $\geq 120,000/mm^3$; 50% for wbc count between 3999 and $2500/mm^3$ and/or platelet count between 119,999 and $75,000/mm^3$; and temporary suspension of treatment for wbc count $< 2500/mm^3$ and/or platelet count $< 75,000/mm^3$. Hemoglobin measurements and wbc and platelet counts were performed before each dose, except on Days 2–5 of the first course of 5-FU.

Assessment of Response

At the time of entry in the study, a clinical examination was performed to measure and record any assessable tumor parameters. Abdominal masses and liver edge that were palpable under the costal margin were measured in centimeters and a map was drawn outlining the assessable tumor. At the same time, each patient had a routine biochemical examination, a liver scan and, if primary or recurrent tumor was present in the stomach, an upper gastrointestinal X-ray. Endoscopic examination was mandatory if tumor in the stomach was the only assessable parameter. Response to treatment was defined by the following criteria: *complete response (CR)*—the disappearance of all signs and symptoms of disease; and *partial response (PR)*—a $\geq 50\%$ reduction in the sum of the product of the two largest perpendicular diameters of all measurable lesions. Liver metastases were used as indicator lesions only if proven by histologic or direct surgical assessment. Objective response was judged to have occurred when: (*a*) liver edge was initially measurable at least 5 cm from the costal margin and there was a decrease by at least 30% in the sum of measurements on the right and left midclavicular line and on the median line and/or (*b*) significant decrease in the diameter of liver lesions on the liver scan, from an initial diameter > 3 cm. Objective improvement (OI) was defined as tumor shrinkage > 25% but < 50%. In addition, it was required that all of the above-mentioned therapeutic effects take place a minimum of 2 months from the beginning of treatment. Stable disease was defined as lack of growth of indicator lesions or volume changes < 25% for at least 2 months. Progressive disease (PD) was defined as a > 25% increase in any measurable lesion or the appearance of new areas of malignant disease.

Direct clinical examination was performed every month to assess therapeutic effects and to record toxicity. Liver and renal function tests were periodically repeated. Radiographic, endoscopic, and radioisotopic investigations were repeated every 2 months to assess clinical response. If the indicator lesion was primary or recurrent tumor in the stomach, evaluation of response required a repeated endoscopic examination, unless contrast radiography clearly showed PD. Duration of response was calculated from the beginning of treatment until objective proof of tumor regression was obtained. Survival was calculated from the beginning of treatment until death.

Statistical comparison of responses between the two treatments was performed by the chi-square method. Durations of response and survival were evaluated by the Kaplan and Meier method (4). Differences between distributions were tested using the Cox-Mantel test (5).

RESULTS

Toxic Effects

Analysis of nonhematologic toxic effects showed that they were more frequent in patients receiving 5-FU; nausea, vomiting, diarrhea, and stomatitis were observed in 58%, 41%, 29%, and 50% of the patients. These symptoms were generally mild to moderate and were generally observed after the initial 5-day course; occurrence was unusual during subsequent weekly administration of the drug. These gastrointestinal toxic effects were observed in a much lower percent of patients who received MFC (nausea and vomiting, 20%; diarrhea, 12%; and stomatitis, 8%). They were always mild and easily controllable with antiemetics. Alopecia was rare in all patients.

Analysis showed that hematologic toxicity was more frequent and severe with MFC than with 5-FU alone. The percents of observed episodes with 5-FU were lower than with MFC in reference to grade 1 (38% vs 70%) and grade 2 (0 vs 12%) leukopenia, and to grade 1 (25% vs 70%) and grade 2 (4% vs 40%) thrombocytopenia. Neither irreversible hematologic toxicity nor complications of myelosuppression (sepsis or bleeding) were observed. As a consequence of the more frequent hematologic toxicity with MFC, the percent of optimal dose administered to the patients was higher with 5-FU. The average percent of optimal 5-FU dose was high and stable during the first 6 months of treatment (98%, 100%, 100%, 97%, 97%, and 93%); with MFC, there was a tendency toward a progressive decrease (95%, 84%, 81%, 74%, 76%, and 69%).

Therapeutic Results

Four of the 53 patients entered in the study were considered to be nonevaluable for therapeutic response; one patient in each arm was lost to clinical controls before the first evaluation, and one patient in each arm died within the first 2 months, before the first assessment of response was performed.

A total of 24 patients who received 5-FU and 25 who received MFC were evaluable. In the 5-FU arm, 14 patients showed PD, three had no change, two had OI, four had PR, and one had CR. Thus, five of 24 patients treated with 5-FU showed an objective remission (21%). In the MFC arm, 13 patients showed PD, five had no change, none had OI, six had PR, and one had CR. Thus, seven of 25 patients treated with MFC showed an objective remission (28%). The difference in objective response rates between the two treatment arms was low and not statistically significant.

There was one CR in each treatment group. One patient treated with MFC achieved complete normalization of previous radiologic and endoscopic evidence of an inoperable tumor in the stomach, with multiple negative biopsies in the site where macroscopic and histologic evidence of tumor had previously been found. Second-look laparotomy was not performed. One patient treated with 5-FU had complete disappearance of metastatic lymph nodes in the left supraclavicular region.

Median duration of response was practically identical between 5-FU (16 weeks; range, 8–26) and MFC (17 weeks; range, 8–33). Median survival was slightly longer with MFC (43 weeks) than with 5-FU alone (39 weeks). The difference was not statistically significant. Responding patients (CR plus PR) had a longer median survival than patients with OI, no change, or PD (5-FU, 47 vs 39 weeks; MFC, 43 vs 22 weeks). These differences did not reach statistical significance, however.

Seven patients were crossed to MFC after PD or relapse on 5-FU. No objective responses were observed: there was OI in one patient, no change in two, and PD in two. Two patients were nonevaluable because of death before the first evaluation of response.

DISCUSSION

The combination MFC was first proposed by Hoshino et al (6) in L1210 mouse leukemia. In 1972, Ota et al (2) reported a 55% response rate in patients with stomach cancer. Even though the additive role of Ara-C to the activity of the combination is questionable or improbable because this drug is inactive in gastric carcinoma, a number of subsequent studies always utilized combinations including all three drugs to allow a comparative evaluation of results (7–9).

We observed a marked difference in type and degree of toxicity between the two treatments. 5-FU induced more frequent gastrointestinal disturbances, especially during the initial loading dose. With MFC, bone marrow depression was common and was manifested especially by thrombocytopenia, which often required a reduction in dose but rarely a transient suspension of treatment. There were no major complications such as fever or infections due to leukopenia, nor were there hematologic symptoms due to thrombocytopenia. Because there was little or no subjective impact induced by the moderate hematologic toxicity, MFC treatment was usually well-accepted by the patients.

Our data showed that MFC induces a response rate of the same order as that observed with 5-FU alone. There was no statistical difference, and it is impossible to speculate whether the slight increase in response rate observed with MFC compared to 5-FU should be attributed to the combination of mitomycin with 5-FU. Because the increase is so small, a statistical resolution of the problem is impracticable. However, the response rate (28%) obtained in this study with the MFC regimen is quite similar to that (31%) referred to in a study of patients with gastric carcinoma by De Jager et al (7) and higher than that (17%) reported by the Gastrointestinal Tumor Study Group (8). In any case, after the first optimistic report of Ota et al (2), all response rates observed with the MFC combination were at about the level usually expected with 5-FU alone. The present study confirms this observation by a direct prospective randomized comparison.

There was no evidence of any difference in duration of response between MFC and 5-FU. Median survival was higher with MFC than with 5-FU, but the difference was not statistically significant. The length of survival was rather high in both arms of this study and clearly longer than that reported for incurable untreated gastric cancer (10). This fact could be due to the rather high performance status of patients entering this study. It should be noted that patients treated with 5-FU were crossed to MFC when they had PD or relapse. With this sequential approach it was possible to achieve some positive results; OI was observed in one evaluable patient, and no change was observed in two among seven evaluable patients. The activity of MFC in patients pretreated with 5-FU was more clearly shown in the study of De Jager et al (7), in which three of 11 patients pretreated with 5-FU had PR with MFC.

Our data do not confirm the previously reported high response rate obtained by the MFC combination in advanced gastric cancer. It should be noted that our dose regimen of MFC was different from that used by the Gastrointestinal Tumor Study Group (300 mg/m²/day of 5-FU, 1 mg/m²/day of mitomycin, and 50 mg/m²/day of Ara-C, all given on Days 1–5). Although that regimen (8) may be more intensive chemotherapy, the reported response rate was 17% (three responses among 18 patients). However, the MFC regimen we used, even if it is not statistically superior to 5-FU alone, may be one of the regimens useful for the treatment of gastric cancer, since a reasonable response rate with low subjective toxicity and relatively prolonged survival can be expected. MFC can also be useful in patients pretreated with 5-FU. Moreover, our study indicates that any good result obtained with combination chemotherapy in advanced gastric cancer should be controlled by a direct phase III comparison with 5-FU alone. In this regard, the role of MFC compared to the regimen containing 5-FU, doxorubicin, and mitomycin (FAM) deserves further clinical studies. These should take into account that the high response rates reported with FAM in the earlier studies (3,11) seem to be lower in subsequent evaluations or in other studies (12,13).

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Vincristine, BCNU, Doxorubicin, and Prednisone (VBAP) Combination in the Treatment of Relapsing or Resistant Multiple Myeloma: A Southwest Oncology Group Study[1,2]

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SUMMARY

A total of 151 patients with myeloma who had relapsed with or were resistant to melphalan and/or cyclophosphamide (Cytoxan) with prednisone received vincristine, carmustine (BCNU), doxorubicin (Adriamycin), and prednisone (VBAP) at 21-day intervals; 39 patients (25%) responded. Of the 123 patients with relapsing myeloma, 37 (30%) responded. Only two (7%) of the 28 patients with resistant myeloma responded. The regimen was well-tolerated in patients who had not had prior extensive irradiation. Thrombocytopenia was the most frequent form of toxicity. Survival was significantly longer in those patients responding to VBAP (median, 78 weeks) than in those not responding (median, 33 weeks) ($P = 0.002$). VBAP is a safe, effective regimen for patients with myeloma who relapse with alkylating agents.

[Cancer Treat Rep 66:1267–1271, 1982]

Patients with multiple myeloma eventually fail to respond to melphalan and/or cyclophosphamide (Cytoxan) either singly or in combination. An alternative form of therapy is desirable for such patients.

A pilot study from the University of Arizona using carmustine (BCNU) and doxorubicin reported a response in seven of 13 patients in relapse after receiving a melphalan and prednisone combination (1). In a review of 462 patients treated with various combinations of alkylating agents, an increase in both response and survival was reported when vincristine was added to a combination drug regimen. Prednisone had previously been reported as having an additive effect on response rate.

Based on these observations a combination of vincristine, BCNU, doxorubicin (Adriamycin), and prednisone (VBAP) was used in patients with myeloma relapsing with or resistant to adequate trials of melphalan and/or cyclophosphamide with prednisone.

MATERIALS AND METHODS

This report presents an analysis of 151 previously treated patients with late-stage multiple myeloma seen at 28 Southwest Oncology Group (SWOG) institutions between December 1974 and August 1977. All 151 patients had progressive symptomatic myeloma. A previous response to melphalan and/or cyclophosphamide with prednisone had been documented in 123 patients. Response had consisted of a > 75% reduction in myeloma protein ("M") peak. All relapsing patients demonstrated progressive M peak elevations while receiving the therapy that produced previous response; 28 patients had shown

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resistance to prior treatment with increasing M peaks.

Included in this analysis are 28 patients who had received < 90 mg/m² of either BCNU or doxorubicin sometime during the course of prior treatment with melphalan and/or cyclophosphamide.

Tumor mass prior to VBAP was rated as high, intermediate, or low, using criteria previously described (3).

Treatment Regimen

When eligible patients had recovered from the toxic effects of previous chemotherapy, VBAP was begun. Vincristine (1 mg) was administered iv, followed by BCNU and doxorubicin (30 mg/m² each). Prednisone (100 mg) was given orally for 4 consecutive days. Courses were repeated at 21-day intervals if there was no myelotoxicity. Treatment was delayed until the granulocyte count returned to 1500/mm³ and the platelet count returned to 100,000/mm³. For most patients, the delay was ≤ 1 week.

The doses of both BCNU and doxorubicin were decreased by 10 mg/m² if the granulocyte count nadir was < 1000/mm³, if the platelet count nadir was < 100,000/mm³, or in the face of an increasing BUN and/or creatinine.

Prior to each course, an ECG was performed and serum calcium, creatinine, uric acid, and BUN were measured. Anemia, hypercalcemia, and hyperuricemia were treated appropriately during the chemotherapy. An adequate trial was defined as three courses of therapy.

Response Evaluation

Clinical response was based principally on the decrease in the M peak or the myeloma protein production rate. Complete response (CR) was defined as the complete disappearance of the myeloma protein. Partial response (PR) was defined as a decrease of 75%–90% in the serum and/or urine M peak. The patients were rated as improved if there was a 50%–75% decrease in serum myeloma protein. No patient was rated as responsive if there was progression of other clinical indicators such as bone lesions, hypercalcemia, or azotemia.

Comparisons of response rates between subgroups were made using the chi-square test, survival curves were estimated using the method of Kaplan and Meier (4), and comparisons of survival duration curves were made using Gehan's generalization of the Wilcoxon test (5).

RESULTS

Five patients (3%) had CR, 20 (13%) had PR, and 14 (9%) were improved, for an overall response rate of 25% (39 responses among 151 patients). Twenty patients (13%) showed no change in the M peak, while 92 (62%) showed evidence of increasing disease. The response rate in relapsing patients was 30% (37 responses among 123 patients). There were only two responders (7%) among the 28 patients who had shown no response to previous therapy.

In the 39 responding patients a detectable decrease in the M peak did not occur until the patient had received at least three courses of VBAP. Thus, it was determined that three courses was the minimum number for adequate trial; 122 patients received at least three courses. The response rate in this adequately treated group was 32% (39 responses among 122 patients).

Of the 29 patients who did not receive an adequate trial of VBAP, 18 died, three refused further therapy, seven did not complete the trial due to toxic effects, and one was lost to followup before three courses were given.

The small amounts of BCNU or doxorubicin that 20 relapsing patients had received prior to VBAP did not significantly influence the response rate (table 1). However, the degree of response was notably

TABLE 1.—VBAP in relapsing or resistant myeloma: response*

	No. of patients treated	No. of responders (%)
Previous responders (relapse)	123	37 (30)
M–C	103	32 (31)
M–C + B or A	20	5 (25)
Previous nonresponders (resistant)	28	2 (7)
M–C	20	2 (10)
M–C + B or A	8	0 (0)
All patients	151	39 (25)

*M = melphalan, C = cyclophosphamide, B = BCNU, and A = doxorubicin.

TABLE 2.—VBAP in relapsing or resistant myeloma: response by type of M peak*

Protein	No. of patients	No. responding (%)	Response				
			CR	PR	IMP	NR	IND
IgG	65	17 (26)	2	6	9	12	30
IgA	37	5 (14)	0	2	3	5	27
BJ	32	12 (38)	1	11	0	2	23
IgG + BJ	15	4 (27)	1	1	2	1	11
IgD	1	0 (0)	0	0	0	0	1
Ig unknown	1	1 (100)	1	0	0	0	0
Total	151	39 (25)	5	20	14	20	92

*IMP = improvement, NR = no response, IND = increasing disease, and BJ = Bence Jones proteinuria.

TABLE 3.—SWOG myelotoxicity criteria

Grade	Nadir	
	Granulocyte count (cells/mm³)	Platelet count (cells/mm³)
1	2000–2499	75,000–99,999
2	1000–1999	50,000–74,999
3	500–999	25,000–49,999
4	< 500	< 25,000

different between the two groups. None of the patients who had relapsed after receiving prior BCNU or doxorubicin had CR, 10% had PR, and 5% were improved. Relapsing patients not previously treated with BCNU or doxorubicin had a 5% CR rate and a 17% PR rate, and 9% improved.

None of the patients in this study had low tumor mass. Ten (32%) of 31 patients with intermediate mass responded, and 29 (24%) of 120 patients with high tumor mass responded.

Table 2 shows response correlated with myeloma protein type. There was a significant difference in response rates in patients with IgA serum peaks and Bence Jones proteinuria. There was no significant difference in response rates among any of the other groups.

Toxicity

Myelotoxicity was recorded in 60 (40%) of the 151 patients (*see* tables 3 and 4). Thrombocytopenia was found in 46 patients (30%) but was reported to be a severe clinical problem in only ten (7%). There was one drug-related death. Nine of the 11 patients who had severe myelotoxicity had received radiotherapy to more than three sites prior to VBAP.

One patient had severe nausea and vomiting after the first course of VBAP and refused further therapy. Gastrointestinal toxicity of a significant degree was quite rare.

Two patients with increasing disease had a > 50% decrease in creatinine clearance after three courses of VBAP. It was not clear if this was due to drug toxicity or a complication of advancing disease. Both patients had light-chain disease.

One patient developed atrial fibrillation, and one showed marked ST-segment changes on ECG after the third course of VBAP. Both patients had advancing disease and received no further therapy; they had received only 90 mg/m² of doxorubicin.

Survival

Percentiles of survival from start of VBAP therapy were determined in 150 of the 151 patients in

TABLE 4.—VBAP in 151 patients with relapsing or resistant myeloma: myelotoxicity

Myelotoxicity	Grade				Fatal
	1	2	3	4	
Granulocytopenia	7	6	0	1	0
Thrombocytopenia	23	10	7	1	1
Pancytopenia	2	1	0	1	0
Total	32	17	7	3	1

this study. The median overall survival was 33 weeks, with 111 patients having died at the time of this analysis.

Objective response to VBAP was strongly related to survival. Of the 122 patients who had received enough treatment to have had a chance at response, the 39 responding patients had a median survival of 78 weeks compared with only 33 weeks for patients with no response. The survival curves for these two groups, as shown in figure 1, were significantly different ($P = 0.002$).

The original date of diagnosis was available; therefore, it was possible to calculate percentiles of survival from that date. The overall median survival was 166 weeks. Patients having received no previous BCNU or doxorubicin had significantly longer survival than those who had. Median survival times were 186 and 125 weeks, respectively ($P = 0.02$).

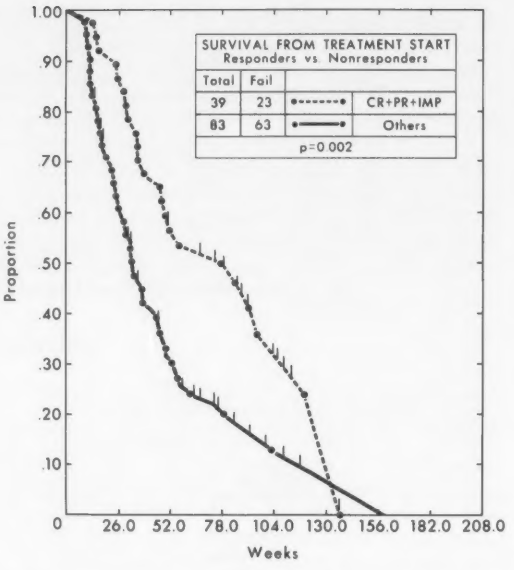

FIGURE 1.—SWOG-7432: survival in patients with relapsing or resistant myeloma treated with VBAP.

DISCUSSION

In previous SWOG studies (2), about 60% of the patients with untreated multiple myeloma achieved remissions as defined by a ≥ 75% reduction in M peak or myeloma protein production rate. Using these same criteria, 16% of relapsing or resistant patients (25 of 151) responded to VBAP. An additional 14 patients (9%) showed a > 50% reduction in M peak and are included as responders in this evaluation. All 14 patients had been rated as responders in previous SWOG protocols and subsequently had shown at least a doubling of the M peak while receiving therapy, making them eligible for VBAP. As a result of VBAP, all 14 patients had an M peak or production rate reduction to the same level or below that at which they had been previously rated as responsive.

The response rate of 30% in patients with myeloma who had responded previously but were currently refractory was encouraging and demonstrates the utility of VBAP for some patients with progressive myeloma. Alexanian et al (6) reported that 80% of previously responding patients followed without chemotherapy until relapse responded to the resumption of melphalan and prednisone. Eventually, this group of responders failed to respond to continued melphalan and prednisone. In this relapsing group, Presant and Klahr (7) reported a 36% response (five responses among 14 patients) to a combination of doxorubicin (Adriamycin), BCNU, cyclophosphamide, and prednisone (ABC-P), using response criteria similar to those used in this study.

Adequately treated patients responding to VBAP showed a prolongation of median survival of 10 months over adequately treated nonresponders. Using ABC-P, Presant and Klahr (7) reported an increase in median survival of 6 months in responders versus nonresponders.

This study also confirms the efficacy of BCNU and doxorubicin combinations as reported in the pilot study reported by Alberts et al (1). While that study was small, a higher response rate was reported. The investigators did not employ a dose reduction of myelosuppressive drugs; patients were supported with granulocyte and platelet transfusions. The current study employed dose reductions. Therefore, there could have been less intensive remission induction in this study.

Using cyclophosphamide, BCNU, and prednisone, Kyle et al (8) reported a response rate of 17% (seven of 42 patients) in patients with myeloma resistant to melphalan. In that study, 7% (three of 47 patients) of the patients with myeloma resistant to melphalan responded to cyclophosphamide and prednisone without BCNU. The objective responders had significantly longer survival than nonresponders (31.0 vs 9.4 months; $P < 0.02$). Whether the apparent increase in response to BCNU and doxorubicin is due to the addition of doxorubicin to BCNU or to other factors such as dose scheduling or patient population cannot be determined from the available data.

The response of two (7%) of 28 patients with myeloma initially resistant to melphalan and cyclophosphamide is not impressive. In the pilot study, initial nonresponders to melphalan were also observed to be resistant to BCNU and doxorubicin (1). Since both studies have reported sensitivity or resistance to BCNU and doxorubicin (with or without vincristine and prednisone) in similar patient categories, it appears that a yet undescribed kinetic or biochemical factor distinguishes responders from nonresponders. Neither doxorubicin nor bleomycin alone has produced promising results in resistant myeloma (9). Although response to VBAP was impressive in one of the previously nonresponding patients, some other treatment regimen for these patients must be sought.

Prior exposure to BCNU or doxorubicin did reduce the rates of CR and PR to VBAP. BCNU or doxorubicin had been given prior to VBAP as part of maintenance protocols. This suggests the acquisition of resistance to these drugs at a time when the patient was considered to be in remission.

VBAP was tolerated in most patients, even those with late-stage disease. There was one drug-related death. Prior chemotherapy did not correlate with toxicity. There was some indication of a correlation between severity of disease and toxicity. The most significant finding was the correlation of severe toxicity to the previous amount of radiation therapy. In patients who had received radiation therapy to fewer than three sites, VBAP was well-tolerated.

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Phase III Study of Intermittent Carmustine (BCNU), Cyclophosphamide, and Prednisone Versus Intermittent Melphalan and Prednisone in Myeloma[1,2]

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SUMMARY

A prospective randomized trial in patients with previously untreated multiple myeloma was performed comparing carmustine (BCNU), cyclophosphamide, and prednisone (BCP) to melphalan (Alkeran) and prednisone (AP). Induction response rates, remission duration, and survival were similar with both regimens. Hematologic toxicity was greater with AP. Crossover studies in patients who relapsed did not illustrate any significant activity with either drug treatment program. Therefore, BCP can be utilized as initial therapy in myeloma because of comparable remission rates and less hematologic toxicity.

[Cancer Treat Rep 66:1273–1277, 1982]

The intermittent melphalan (Alkeran) and prednisone (AP) regimen has been commonly used as induction therapy for multiple myeloma. Response rates of approximately 50% have been substantiated by many investigators (1,2) and the drug regimen is well-tolerated. Despite these results, it is necessary to improve survival by finding better induction and maintenance therapy. Encouraging preliminary results with carmustine (BCNU) and cyclophosphamide (3,4) prompted the Eastern Cooperative Oncology Group (ECOG) in 1972 to study (EST 4472) the combination of BCNU, cyclophosphamide, and prednisone (BCP) versus AP. Treatment groups were evaluated in terms of response rates, toxic reactions, crossover response rates, and survival.

METHODS

Untreated patients with multiple myeloma as judged by the guidelines of the Chronic Leukemia-Myeloma Task Force (5) were eligible if measurable

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disease was present (a monoclonal protein or plasmacytoma). Patients were stratified into good-risk (hemoglobin ≥ 9 g/100 ml, BUN ≤ 30 mg/100 ml, and calcium ≤ 12 mg/100 ml) and poor-risk (failure to meet good-risk criteria or expected survival < 6 months) categories. They were randomized to receive either (*a*) 75 mg/m² of BCNU iv over 30 minutes on Day 1, 400 mg/m² of cyclophosphamide by iv push on Day 1, and 75 mg/day of prednisone orally on Days 1–7 in a cycle that was repeated every 4 weeks or (*b*) 8 mg/m²/day of melphalan orally on Days 1–4 and 75 mg/day of prednisone orally on Days 1–7 every 4 weeks. After six courses of therapy, patients were considered to have a partial response (PR) if serum monoclonal protein or plasmacytoma was reduced by 50% or urine monoclonal protein was reduced by 90% (in the presence of stable or improved renal function). Complete response (CR) was defined as the complete disappearance of measurable disease. Responding patients continued to receive the same induction regimen for 2 years. Patients not achieving a response or later failing initial therapy were crossed over to the alternate induction regimen. Survival data include all patients regardless of cause of death. Toxicity was judged according to standard ECOG criteria. Hematologic toxicity was considered severe when wbc counts decreased to < 2000/mm³, granulocyte counts decreased to < 1000/mm³, or platelet counts decreased to < 50,000/mm³.

Tests for differences in discrete or frequency data were done with Fisher's exact test and Zelen's k-2×2 table methodology (6,7). Such data were also evaluated using the linear logistic model and multiple regression analysis (6). The "leaps and bounds" algorithm of Furnival and Wilson (8) was used to select the final regression model, with Mallows' Cp statistics as the selection criteria (9). Survival curves were drawn using the Kaplan-Meier method (10). Medians and survival percentages were determined from the survival curves.

RESULTS

Of 234 patients entered in the study, 188 were analyzed; 142 were evaluable and 46 were unevaluable, mostly because they lacked measurable disease or refused therapy. The group of unevaluable patients differed from the study group only in that it included fewer patients with hypercalcemia (35% as compared to 62%) and more blacks (36% as compared to 16%). Unevaluable patients did not differ in terms of other prognostic factors and had no statistically significant difference in survival. As shown in table 1, patient characteristics, except for sex, were equally distributed between the two treat-

TABLE 1.—Distribution of cases analyzed by on-study characteristics

Category	Level	No. of patients (%)		
		BCP	AP	Total
Initial performance status	0	22 (24)	15 (19)	37 (22)
	1	32 (36)	29 (36)	61 (36)
	2	11 (12)	13 (16)	24 (14)
	3	19 (21)	19 (23)	38 (22)
	4	6 (7)	3 (6)	9 (6)
	Not reported	6	11	17
Age	< 60 yrs	29 (31)	31 (34)	60 (32)
	60–70 yrs	37 (39)	33 (37)	70 (38)
	≥ 70 yrs	29 (31)	26 (29)	55 (30)
	Not reported	1	2	3
	Median age	64 yrs	63 yrs	64 yrs
Sex	Male	70 (73)	54 (59)	124 (66)
	Female	26 (27)	38 (41)	64 (34)
Race	White	79 (84)	76 (84)	155 (84)
	Black	15 (16)	14 (16)	29 (16)
	Not reported	2	2	4
Risk status	Good	62 (65)	54 (59)	116 (62)
	Poor	34 (35)	37 (41)	71 (38)
	Not reported	0	1	1
Prior surgery	No	85 (89)	84 (91)	169 (90)
	Yes	11 (11)	8 (9)	19 (10)
Prior radiotherapy	No	83 (86)	74 (80)	157 (84)
	Yes	13 (14)	18 (20)	31 (16)

TABLE 2.—Response rates for all evaluable patients by risk status

	No. of patients	
	BCP	AP
All patients		
CR	1	4
PR	34	27
No response	35	41
Response rate*	35/70 (50%)	31/72 (43%)
Good risk		
CR	0	3
PR	24	17
No response	24	23
Response rate*	24/48 (50%)	20/43 (47%)
Poor risk		
CR	1	1
PR	10	10
No response	11	18
Response rate*	11/22 (50%)	11/29 (38%)

*No. of CR + PR/No. of evaluable patients.

ment groups. Table 2 lists the response rates for the two therapies. For all patients, there was no significant difference in the response rates between BCP and AP. BCP treatment among 70 evaluable patients resulted in 35 CR plus PR, or a response rate of 50%, which was not statistically different from the 31 CR plus PR (43%) among 72 evaluable patients given AP treatment. There was one CR with BCP and four with AP. Response rates for good-risk and poor-risk patients demonstrated no significant differences between the two treatments. Performance status, age, BUN, calcium, sex, and race did not influence the response rates between the two treatment arms.

The toxicity of each treatment arm was categorized as severe or none to moderate. Table 3 shows the hematologic toxicity of BCP and AP according to pretreatment risk status. There were 21 patients excluded from this analysis because of lack of toxicity data. Although poor-risk patients had a higher percent of hematologic toxicity with AP as compared to good-risk patients, there was no statistical evidence of treatment-risk interaction ($P = 0.44$). However, when the group was analyzed as a whole, a statistically significant higher rate of severe hematologic toxicity occurred with AP than with BCP ($P = 0.03$). Gastrointestinal toxicity as judged by nausea, vomiting, or diarrhea was not greater in either treatment arm.

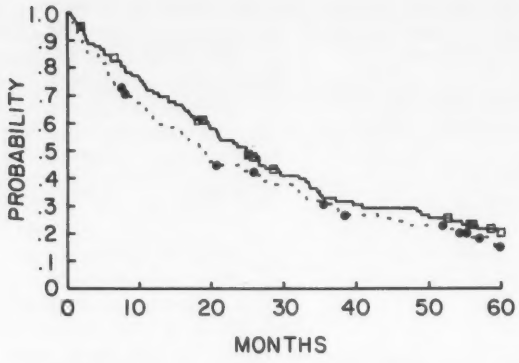
Survival was determined from the date of entry in the study and is shown in figure 1. Of 188 patients, 82% have now died. There was no difference in survival between the BCP- and AP-treated patients; median survival for the entire group was 21 months. Not illustrated is an improved median survival of 28 months for ambulatory patients as compared to 12.5 months for nonambulatory patients ($P = 0.01$), as well as improved survival in the group with better performance status and lower BUN. Initial calcium levels did not influence survival. There was no difference in survival between patients in the two treatment arms when separately analyzed by age, risk status, calcium, or BUN. The duration of response was evaluated from the onset of response and is shown in figure 2. The median length of response was 18 months for BCP and 24 months for AP; this difference was not statistically significant ($P = 0.88$).

Crossover data were evaluable in 47 patients. Only three of 28 patients receiving BCP in relapse responded as did three of 19 patients receiving AP in relapse; this difference was not significant.

DISCUSSION

The comparison of the BCP combination to the AP combination naturally followed the encouraging preliminary results with BCNU and cyclophosphamide used as single agents (4). In addition, 25%–30% of the patients who failed after melphalan treatment were reported to respond to yet another alkylating agent, cyclophosphamide (3). Furthermore, the combination of more than one alkylating drug given concurrently or sequentially was being studied in several cooperative groups (11–13).

In the study reported here, previously untreated patients with myeloma and measurable disease were randomized to receive BCP or AP. There was no difference in response rates between the BCP and AP groups. Furthermore, the duration of response

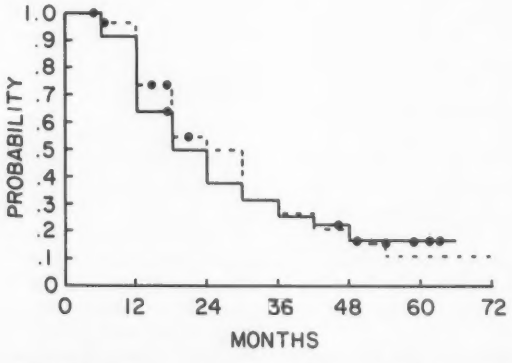


TREATMENT	ALIVE	DEAD	TOTAL	MEDIAN
—— BCP	19	77	96	24.6
----- AP	14	77	91	18.8

FIGURE 1.—Survival of patients according to induction treatment arm.

for the two groups was the same, which suggests that no greater tumor destruction was afforded by either therapy. In addition, survival of responding and nonresponding patients was unaffected by initial treatment choice. The response rate for the BCP arm in this trial is comparable to that initially reported by Cohen et al (11) for the same regimen. The use of AP in this trial resulted in more hematologic toxicity; however, as already shown, toxicity did not adversely affect duration of response or survival.

BCP can be considered as an acceptable alternative to the commonly used AP combination in the initial treatment of myeloma. In addition, the iv route is a more reliable method of delivering chemotherapy as compared to the oral route. Since BCP has less hematologic toxicity, one could consider adding other induction drugs to this regimen in future trials. Crossover data showed few secondary responses with either regimen, which suggests lack of cross-reactivity among alkylating agents. However, patients receiving crossover drugs in this study



TREATMENT	CONT	FAIL	TOTAL	MEDIAN
—— BCP	7	28	35	18.0
----- AP	6	22	28	24.0

FIGURE 2.—Duration of initial response according to induction regimen.

TABLE 3.—Degree of hematologic toxicity by treatment and risk status

Risk status	Treatment	No. of patients (%) by degree of toxicity	
		None to moderate	Severe
Good	BCP	50 (85)	9 (15)
	AP	37 (77)	11 (23)
Poor	BCP	23 (82)	5 (18)
	AP	17 (55)	15 (45)

might have had far-advanced disease; therefore, crossover data are not analogous to data from the use of sequential or concomitant alkylating therapy. On this point, Case et al (14) have reported that the combination of BCNU, cyclophosphamide, vincristine, melphalan, and prednisone is significantly more effective in remission induction without producing excessive toxicity. A randomized trial of what is still considered to be standard therapy (AP) versus the concomitant use of alkylating treatment has been initiated to address the value of combined alkylating agent therapy in myeloma.

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Randomized Study Comparing Doxorubicin, Cyclophosphamide, Vincristine, Methotrexate With Leucovorin Rescue, and Cytarabine (ACOMLA) With Cyclophosphamide, Doxorubicin, Vincristine, Prednisone, and Bleomycin (CHOP-B) in the Treatment of Diffuse Histiocytic Lymphoma[1,2]

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SUMMARY

Two combination chemotherapy programs, ACOMLA (doxorubicin, cyclophosphamide, vincristine, methotrexate with leucovorin rescue, and cytarabine) and CHOP-B (cyclophosphamide, doxorubicin, vincristine, prednisone, and bleomycin), were evaluated in 29 prospective randomized patients with advanced diffuse histiocytic lymphoma. A complete response was achieved in 13 of the 15 patients (87%) treated with CHOP-B and in nine of the 14 patients (64%) treated with ACOMLA. The overall complete response rate was 75%. Two patients treated with ACOMLA and none of the CHOP-B-treated patients have relapsed. Median followup is 32 months for ACOMLA patients and 26 months for CHOP-B patients. Actuarial freedom from relapse at 2 years is 49.9% for ACOMLA and 93.3% for CHOP-B ($P = 0.04$). Toxicity was substantial, with eight nonfatal episodes of sepsis and three drug-related deaths. There have been no central nervous system relapses. Although patients treated with CHOP-B have a better response rate, the small numbers of patients treated to date preclude definitive conclusions.

[Cancer Treat Rep 66:1279–1284, 1982]

Diffuse histiocytic lymphoma is a potentially curable malignancy. Several regimens have produced complete response rates from 41% to 69% including C-MOPP (cyclophosphamide, mechlorethamine, vincristine, procarbazine, and prednisone); CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone); and CHOP-B (cyclophosphamide, doxorubicin, vincristine, prednisone, and bleomycin) (1–3). Occasional modifications of these drug schedules have also been successful (4).

In 1972, Levitt et al (5) retrospectively evaluated 15 patients with reticulum cell sarcoma treated with COMLA (cyclophosphamide, vincristine, metho-

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trexate with leucovorin rescue, and cytarabine). Nine patients had a complete response. COMLA employed antimetabolites when cells theoretically would have been in logarithmic phase growth following the high fraction cell kill from cyclophosphamide and vincristine. More recent laboratory data have indicated a dose- and schedule-dependent synergism between methotrexate and cytarabine in L1210 leukemia cells and human myeloblasts (6).

We have previously reported our results using this drug program to treat 30 patients with diffuse histiocytic lymphoma. Twelve patients had a complete response, but six of these patients relapsed (7). A recent series of 42 patients treated at the University of Chicago with COMLA had better results; there were 20 (48%) durable complete remissions (8).

In 1975, we modified the COMLA regimen by adding doxorubicin, an efficacious agent for the treatment of diffuse histiocytic lymphoma (2–4). A randomized trial in stage III and IV diffuse histiocytic lymphoma was initiated to compare this new antimetabolite program, ACOMLA, to the commonly used drug combination CHOP-B.

PATIENTS AND METHODS

Between May 1976 and September 1980, 29 consecutive patients with advanced stage (III or IV) diffuse histiocytic lymphoma were entered in the protocol after obtaining informed consent. Pathology was reviewed at our institution. Patients were not eligible if they had received prior chemotherapy. Prior radiotherapy did not exclude patients from the protocol. Measurable disease, an absolute granulocyte count $> 500/mm^3$, a platelet count $> 50{,}000/mm^3$, and age > 16 years were required for eligibility. Radiotherapy was used to treat only life-threatening sites of disease, and patients were excluded from the study if all the known sites of disease had received radiation. No patients were excluded for medical conditions or a poor Karnofsky status.

All patients were clinically staged using the Ann Arbor classification. Evaluation included cbc, liver and renal function tests, chest X-ray, bilateral bone marrow biopsies and aspirates, lymphangiography with iv pyelogram, gallium scan, and liver-spleen scans. Abdominal ultrasound, computerized axial tomography, and gastrointestinal series were obtained in patients with intra-abdominal involvement or gastrointestinal symptoms. All patients who had bone marrow involvement had a lumbar puncture for cytologic examination.

Those patients with stage III and IV disease were randomized to one of two treatments: ACOMLA or CHOP-B. ACOMLA consisted of cyclophosphamide (1.0 g/m^2) and doxorubicin (hydroxydaunorubicin) (40 mg/m^2) given on Day 1; vincristine (2.0 mg) given on Days 1, 8, and 15; and methotrexate (120 mg/m^2) followed by cytarabine (cytosine arabinoside) (300 mg/m^2) given 1 hour later on Days 22, 29, 36, 43, 50, 57, 64, 71 (fig 1). Leucovorin (25 mg) was administered orally 24 hours after the methotrexate and was continued every 6 hours for six doses. Three cycles, each lasting 3 months, were administered. CHOP-B consisted of cyclophosphamide (1.0 g/m^2), doxorubicin (40 mg/m^2), and vincristine (2.0 mg) given on Day 1; bleomycin (15 units) given on Days 1 and 5; and prednisone (100 mg/m^2) given daily for the first 5 days. The cycle was repeated every 4 weeks, for a total of nine cycles. The total treatment time for both drug programs was 9 months. Drug dose was reduced by 33% if the wbc count was $< 2500/mm^3$ during the methotrexate and cytarabine phase of ACOMLA. If the nadir granulocyte count was $< 300/mm^3$ after cyclophosphamide and doxorubicin for either regimen, the subsequent drug dose was reduced by 33%.

One month after completion of therapy all patients were restaged using the same clinical procedures described for initial evaluation. Previous sites of disease which were clinically detectable were rebiopsied. Patients were followed regularly at 2-month intervals until 24 months after completion of therapy. Followup intervals were then increased to between 4 and 6 months. Complete response was defined as the total disappearance of all known disease for a duration of 2 months. Partial response was defined as a 50% reduction in tumor parameters for > 2 months.

Survival and freedom from relapse are calculated from the date of protocol entry and the date of response, respectively, using the Kaplan-Meier method. The curves were compared using the Wilcoxon test.

RESULTS

The clinical characteristics are summarized in table 1. Sites of involvement in the stage IV patients included gastrointestinal tract (four patients), lung (four), bone (three), bone marrow (three), pleura (two), and liver, pancreas, abdominal peritoneum, prostate, and breast (one each).

All patients achieving a complete response were clinically free of disease within 2 months of treatment initiation. Overall, 75% of the patients achieved a complete remission. Thirteen of the 15 patients treated with CHOP-B achieved a complete remission (87%). None of these patients has relapsed. Of

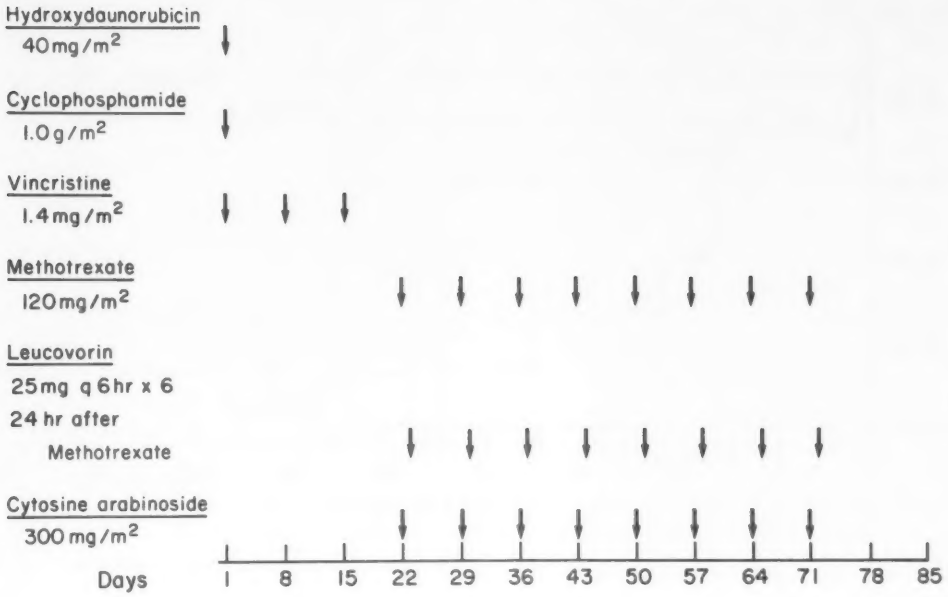

FIGURE 1.—ACOMLA drug regimen schedule.

TABLE 1.—Clinical characteristics of patients with diffuse histiocytic lymphoma: ACOMLA vs CHOP-B

	ACOMLA group*		CHOP-B group†	
	No. (%) of patients	No. (%) with complete response	No. (%) of patients	No. (%) with complete response
Total	14	9 (64)	15	13 (87)
Stage				
IIIA	2 (14)	2 (100)	5 (33)	5 (100)
IIIB	1 (7)	0	0	—
IVA	8 (57)	5 (63)	7 (46)	6 (86)
IVB	3 (21)	2 (66)	3 (20)	2 (66)
Gastrointestinal involvement	3 (21)	1 (33)	3 (20)	3 (100)
Bone marrow involvement	3 (21)	0	0	—
Tumor > 10 cm	2 (14)	1 (50)	3 (20)	2 (66)
Concomitant radiation therapy	6 (43)	4 (66)	6 (40)	5 (83)

*Mean age: 57 ± 17.5 yrs.
†Mean age: 59 ± 12.6 yrs.

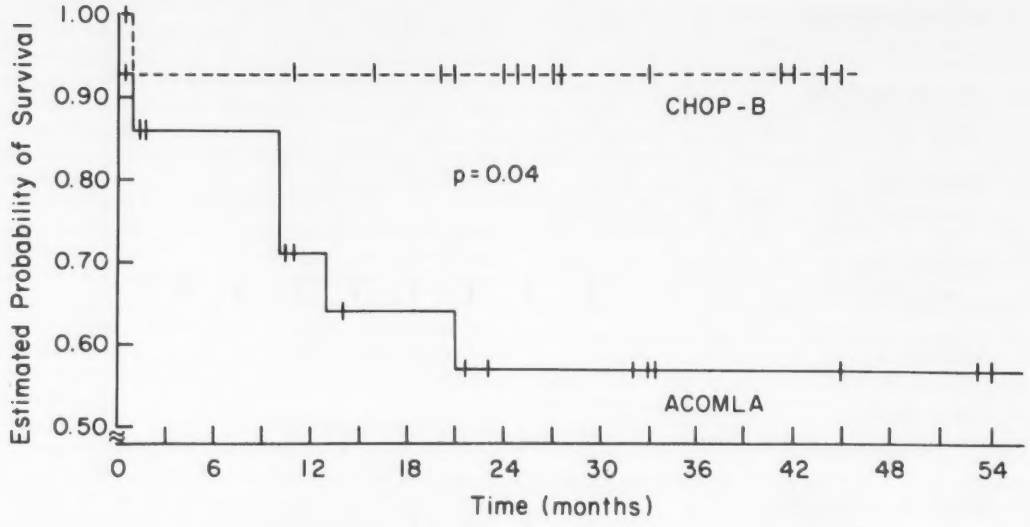

FIGURE 2.—Survival of patients with diffuse histiocytic lymphoma treated with ACOMLA or CHOP-B.

the 14 patients treated with ACOMLA, nine have had complete response (64%); two of these patients subsequently relapsed at 20 and 13 months. The difference between the percent complete remission was not significant ($P = 0.2$). One relapsing patient has achieved a second complete remission to CHOP-B. The average survival for the seven remaining patients who did not respond to therapy was 8 months. The number of patients is too small to accurately evaluate prognostic indicators. However, none of the three patients with bone marrow involvement achieved a complete response. Relapse in the central nervous system has not been documented in any patient. The survival and freedom from relapse curves are shown in figures 2 and 3, respectively.

Drug toxicity was substantial. Three drug-related deaths from sepsis occurred during induction therapy; two patients had received ACOMLA. Of the three patients dying from sepsis, one had gastric involvement with lymphoma, developed a perforation 7 days after his first drug treatment, and died from sepsis and acute respiratory distress syndrome 1 week later; the other two patients had extensive stage IV disease and a Karnofsky status of 10. None of these patients had bone marrow involvement or prior radiation therapy. Eight febrile episodes occurred during the first course of chemotherapy at approximately 10–14 days: four in each treatment arm. In all cases, the wbc count was $< 1000/mm^3$. The platelet count was never $< 75,000/mm^3$. There were no pulmonary complications associated with bleomycin. One patient developed congestive heart failure and angina pectoris while receiving CHOP-B, at a total doxorubicin dose of 240 mg/m^2. Nausea and vomiting were experienced by all patients during methotrexate and cytarabine administration. Cumulative doses of cyclophosphamide and doxorubicin were reduced by 50% in four patients receiving CHOP-B; only one patient was an induction failure. The dose of these drugs was also reduced by 50% in four patients treated with ACOMLA; none of these patients were induction failures. There was no hematologic toxicity following the methotrexate and cytarabine treatment.

Three patients developed second malignancies. Squamous cell carcinoma of the lung and prostatic carcinoma were discovered in two elderly patients treated with CHOP-B within 1 year of achieving a complete remission. Both are alive and free of lymphoma. Acute myelogenous leukemia was diagnosed 1 month after completion of ACOMLA in a patient who had a marrow involvement with diffuse histiocytic lymphoma at entry in the study. Re-evaluation of the pathology confirmed this unusual simultaneous occurrence.

Radiotherapy, which was never given to all sites of disease in any patient, was employed in various doses for potentially catastrophic lesions such as involved gastrointestinal tract or weight-bearing

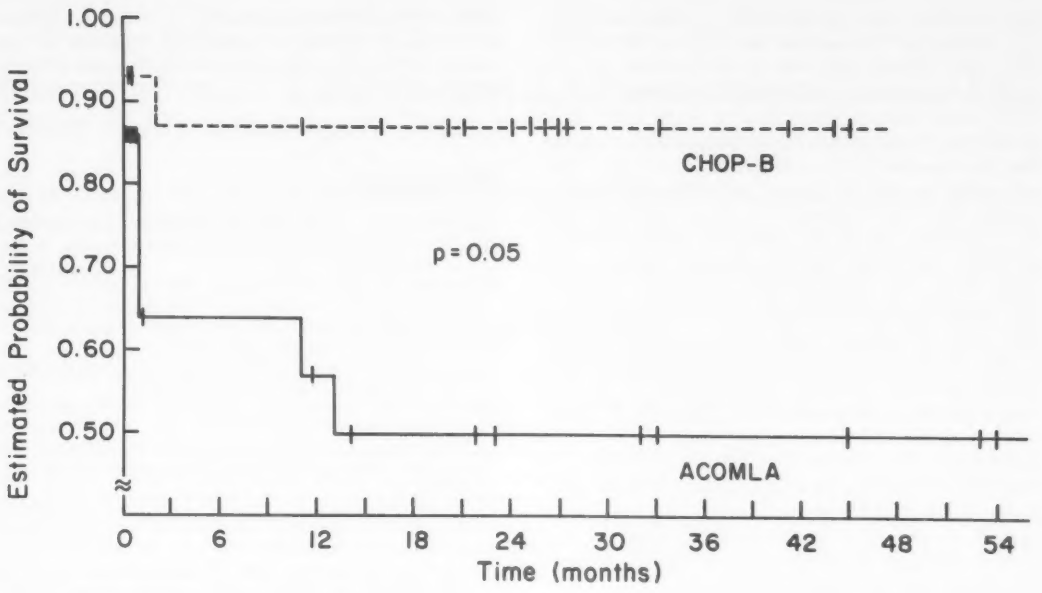

FIGURE 3.—Disease-free survival of patients with diffuse histiocytic lymphoma treated with ACOMLA or CHOP-B.

bones. The radiation therapy generally preceded drug treatment by 1–3 weeks, with an average of 2000 rads. Radiotherapy was utilized in six patients treated with CHOP-B (stomach, two; pelvis, two; and spleen and colon, one each). Five of these patients subsequently had a complete response to the chemotherapy. However, seven of the remaining eight patients treated with CHOP-B who were not given radiotherapy also achieved a complete response. In the ACOMLA arm, six patients were given radiotherapy (femur, two; pelvis, small bowel, stomach, and testicle, one each). Four of these patients achieved a complete response while only three of the other eight patients treated with ACOMLA who did not receive radiotherapy had a complete response. These differences are not statistically significant.

DISCUSSION

The treatment of advanced diffuse histiocytic lymphoma has improved significantly in the past 10 years. Early combinations of low-dose cyclophosphamide, vincristine, and prednisone (CVP) produced a complete remission rate of only 15% (9). The use of high doses of cyclophosphamide and the addition of doxorubicin appeared to increase the complete remission rate. Combinations including C-MOPP, CHOP, HOP (doxorubicin, vincristine, and prednisone), CHOP-B, and BACOP have produced complete remission rates of 41%–69% (1–4). Sweet and Golomb (10), in a recent review of the treatment of diffuse histiocytic lymphoma, note that patients who are carefully restaged clinically and remain relapse-free for at least 2 years appear to be cured of their disease.

The COMLA regimen evolved for several theoretic advantages. The antimetabolites, methotrexate and cytarabine, which kill cells that are synthesizing DNA, were given after tumor lysis from established agents to ensure maximal cell kill from these agents while the tumor was in a rebound of rapid growth. Sequential administration of cytarabine after methotrexate was also shown to kill cells synergistically by enhancing intracellular accumulation of cytarabine (6). In addition, both methotrexate and cytarabine cross the blood-brain barrier and therefore might possibly reduce relapses within the central nervous system. Intracranially transplanted L1210 leukemia will, for example, respond to systemically administered cytarabine in mice (11).

Sweet et al (8) recently reported that among 42 patients carefully restaged there were 23 complete responses and only three relapses. The median dis-

ease-free survival of patients in complete remission was 18 months. The median survival was 33 months. Only one patient relapsed in the central nervous system. A recent study by Skarin and Canellos (12), which used methotrexate (3 g/m^2 iv) in addition to bleomycin, doxorubicin, cyclophosphamide, vincristine, and dexamethasone (M-BACOD), was also quite successful in the treatment of diffuse histiocytic lymphoma. The complete response rate was 80% (45 responses among 56 patients). Seven patients relapsed, one in the central system. Two other patients who were apparently in complete remission also developed central nervous system lymphoma before the scheduled tenth cycle of drug administration. None of our patients had a relapse in the central nervous system. The contrast in central nervous system relapse between the study of Sweet and our study compared to the study of Skarin is interesting. Could it be that the cytarabine is the drug which accounts for this difference? In two separate evaluations from the National Cancer Institute (Bethesda, MD), the percent of central nervous system relapses in patients with diffuse histiocytic lymphoma was 29% and 12% (13,14).

Although patients treated with CHOP-B had a higher complete response rate, the differences in patient populations prevent definitive conclusions. Poor prognostic indicators which have been identified include bone marrow, gastrointestinal tract, or central nervous system involvement, the presence of B symptoms, or a tumor > 10 cm in diameter (15,16). The CHOP-B group is biased for a more favorable response by more stage III patients and an absence of patients with bone marrow involvement.

Drug doses differed in both treatment groups compared to other reported combinations. CHOP-B in the M. D. Anderson Hospital experience utilized 750 mg/m^2 of cyclophosphamide (3). The higher dose in our program may account for our higher response rate. The COMLA combination at both the University of Chicago and Yale University used 1.5 g/m^2 of cyclophosphamide (7,8). The addition of doxorubicin (40 g/m^2) to COMLA with cyclophosphamide dose reduction (1 g/m^2) does not appear to offer any advantage when compared to the University of Chicago series which did not use doxorubicin. Patients in the CHOP-B arm received three times more total doxorubicin (360 mg/m^2), cyclophosphamide (9 g/m^2), and vincristine (54 mg) than the ACOMLA patients. Because the differences between these two treatments were small, it is difficult to interpret the importance of the dose reduction in the ACOMLA program.

Both drug combinations have resulted in similar responses. CHOP-B is easier to administer with fewer clinic visits and less nausea and vomiting. ACOMLA or COMLA remains an excellent regimen for patients with contraindications to large doses of doxorubicin or bleomycin.

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Hexamethylmelamine and Cisplatin in Advanced Ovarian Cancer After Failure of Alkylating-Agent Therapy[1,2]

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SUMMARY

Thirty-eight women with advanced ovarian cancer resistant to alkylating-agent chemotherapy were treated with a combination of hexamethylmelamine and cisplatin. Hematologic toxicity was severe or life-threatening in seven of 13 patients treated with cisplatin at a dose of 100 mg/m² but was acceptable in 25 patients treated at 75 mg/m². Thirty-four percent of the combined group developed peripheral neuropathy and only 8% of those treated at 75 mg/m² developed transient azotemia. Nine complete responses and 12 partial remissions were noted among 38 patients treated, with no obvious advantage for the higher dose of cisplatin. The median duration of remission was 8 months and the overall median survival was 9 months. The response rate of 55% is approximately twice that reported with either agent given alone in similar patient populations and is equal to or better than that reported for more complicated regimens containing these drugs together with other, less active drugs.

[Cancer Treat Rep 66:1285–1290, 1982]

Hexamethylmelamine (HXM) (1–3) and cisplatin (DDP) (4–6) are two of the most active drugs in chemotherapy for ovarian cancer. These are the only drugs with objective response rates > 10% after failure of alkylating-agent chemotherapy (7). The use of these agents in combination is attractive since their major toxic effects differ. The major toxicity of HXM is neurologic whereas that of DDP is renal. Neither drug causes major myelosuppression at usual therapeutic doses. Exacerbation of the moderately severe vomiting induced by DDP can be avoided by delaying the start and limiting the dose of HXM (8). The present trial was a pilot study to identify the maximum doses of these agents which can be safely administered together after failure of alkylating-agent therapy for advanced ovarian cancer and to determine whether the use of the two drugs together may be better than either given alone.

A preliminary study at our institutions showed that the combination of these two drugs with doxorubicin (Adriamycin) was active and tolerable in this patient population, but that no patient was cured by such treatment (8). The response rate with this regimen, which we called "HAD," was 54%, with a median remission duration of 6 months. Doxorubicin was initially included because of evidence that its activity is equal to that of melphalan in patients without prior chemotherapy (9). Melphalan had become the standard alkylating agent used in the US because it is orally administered and produces neither vomiting nor alopecia. Because of new information that doxorubicin has little activity after failure of alkylating agents in ovarian cancer (7,10,11),

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it seemed likely that a combination including only HXM and DDP would be as useful as HAD in such patients. In addition, deletion of doxorubicin would avoid alopecia and reduce myelosuppression.

Having decided that the combination of HXM and DDP was of interest and of potential value, it still remained unclear how much DDP to administer. Data on the optimal DDP dose after failure of alkylating agents are conflicting. In the initial experience of Wiltshaw and Kroner (4), 30 mg/m² given once every 3 weeks was approximately as active as 30 mg/m²/day for 3 days every 3 weeks. Both regimens had response rates of about 27%. Results at the National Cancer Institute (Bethesda, MD) using DDP as a single agent at a dose of 70 mg/m² (response rate, 29%) were not significantly better (3). However, in a later study, Wiltshaw et al (12) obtained a 52% response rate with a dose of 100 mg/m², including two responses among seven patients who had had disease progression with lower doses of DDP. Bruckner et al (13) observed three second responses to 120 mg/m² of DDP among six patients who had responded to 50 mg/m² and then failed, but only one response among 14 who had failed to respond to 50 mg/m². The duration of these responses was not stated, making it difficult to assess the value of the remissions.

Unfortunately, the optimal DDP dose when this drug is given as part of initial combination chemotherapy is also not clear. The only study addressing this problem is that of Ehrlich et al (14) in which approximately 80 patients received 50 mg/m² of doxorubicin and 750 mg/m² of cyclophosphamide monthly with DDP given at either 50 mg/m² once a month or 20 mg/m²/day for 5 days once a month. There was no difference in response rate, response duration, or survival between the two groups, but the cumulative DDP dose was limited to 300 mg/m² in each group. Thus, any advantage that might have occurred by giving more DDP early may have been wiped out by giving no further DDP after the third chemotherapy cycle in the higher-dose treatment program.

The present trial was a pilot study to determine the maximum tolerated dose of DDP with HXM in a patient population with progressive ovarian cancer in spite of alkylating-agent therapy. Based on Wiltshaw's data (4,12), we expected to be able to administer 100 mg/m² of DDP together with HXM. We undertook the present trial to make sure that toxicity was not prohibitive, since experience with this dose in this patient population had been limited. The maximum dose of HXM was determined by gastrointestinal tolerance. In our experience, very few patients can tolerate > 200 mg/m² in divided doses daily, so this dose of HXM was chosen for the current trial (8).

MATERIALS AND METHODS

Eligibility was restricted to women with a histologically proved diagnosis of epithelial ovarian cancer. Tumor had to be beyond the pelvis (stage III or IV) or there had to be unresectable pelvic disease. Progressive tumor despite an adequate trial of alkylating-agent chemotherapy was required, as was duration > 3 weeks from last chemotherapy or irradiation, and tumor which could be measured or at least evaluated for response. Patients were excluded from the study if they had had prior chemotherapy with either HXM or DDP; if they had wbc counts < 4000/μl, platelet counts < 100,000/μl, or serum creatinine > 1.5 mg/dl; or if they were > 75 years old or bedridden 100% of the time (Eastern Cooperative Oncology Group [ECOG] performance status [PS] 4).[3]

The original protocol regimen (HD-100) stipulated the administration of 100 mg/m² of DDP iv on Day 1 with hydration and diuresis. This program began with an iv bolus of 40 mg of furosemide, followed by 2 liters of 5% dextrose in 0.45% saline with 10 mEq of KCl/liter infused over 2 hours. Then 12.5 g of mannitol and 40 mg of furosemide were given iv as successive boluses and 100 mg/m² of DDP was infused over 30 minutes. This was followed by an infusion of 500 ml/hour of 5% dextrose in 0.45% saline with 10 mEq of KCl/liter for 4 hours.

After the first 13 patients, the DDP dose was reduced to 75 mg/m² (HD-75) and a simpler program of hydration and diuresis, which is suitable for outpatient treatment, was used. DDP was then given 30 minutes after beginning a 2-liter infusion of 5% dextrose in 0.45% saline with 10 mEq of KCl/liter given over 2 hours. Furosemide (40 mg) was given as an iv bolus at the start of the infusion, and mannitol (12.5 g) followed by DDP (75 mg/m²) were given as successive iv boluses 30 minutes after the start of the infusion.

HXM (200 mg/m²/day) was given orally in divided doses for 14 days. Therapy was started 8 days after DDP to allow patients to recover from the vomiting induced by DDP before beginning a potentially nauseating oral drug such as HXM. HXM dose was adjusted downward if nausea occurred. Cycles were repeated every 21 days.

Doses were reduced by 33% if, on the day of planned iv drug administration, the wbc count was 3000–4000/μl or the platelet count was 75,000–100,000/μl. Treatment was withheld on a week-by-week basis

[3]ECOG PS: 0 = fully active; 1 = ambulatory with symptoms; 2 = bedridden < 50% of the day; 3 = bedridden > 50% of the day; and 4 = 100% bedridden.

if blood cell counts were below these levels. HXM was given regardless of blood cell counts unless, on Day 14 of a given cycle, the wbc count was < 1500/μl or the platelet count was < 40,000/μl, in which case HXM was discontinued for the remainder of that cycle and all further chemotherapy doses were reduced to 75% of otherwise stipulated levels for the remainder of protocol chemotherapy for that patient.

Complete response was defined as the absence of any evidence of disease in a patient without symptoms or signs related to ovarian cancer. Partial response was defined as a > 50% decrease in the sum of the products of perpendicular diameters of measurable lesions without increase in the size of any lesion, without deterioration in performance status and without a > 10% decrease in dry body weight. When only evaluable disease (ill-defined pelvic masses, ascites, or pleural effusion) was present, an estimated 80% improvement was the criterion for partial remission. Progressive disease was defined as a > 25% increase in the sum of the products of perpendicular diameters of measurable lesions, the development of a new lesion, a > 1-point deterioration in PS on the ECOG scale,[3] or a > 10% weight loss.

Therapy was continued until evidence of progressive disease. Pathologic specimens were reviewed by pathologists at the individual cooperating institutions in this trial, and the findings were correlated with the results.

Patients

Forty-one patients were entered in the trial; three were excluded from further analysis. One of these patients had concurrent metastatic breast cancer. The second patient was lost to followup on Day 3 after receiving only DDP. The third patient never received HXM because of gastrointestinal hemorrhage unrelated to ovarian cancer or its treatment. Of the 38 evaluable patients, 71% were ambulatory (ECOG PS 0 or 1), 42% had received prior radiotherapy, 60% had been treated with other drugs in addition to alkylating agents, and 89% had undergone major surgical procedures for ovarian cancer. The median age was 56 years (range, 32–73). The median interval from diagnosis to entry in this study ranged from 1 to 156 months (median, 12.5). All patients had tumors at least 2 cm in diameter at entry. All patients except three were white. Sixty-three percent of the patients had had no significant weight loss in the 6 months prior to entry, while 16% had had a weight loss > 10%. Extra-abdominal disease was present in 29%. The institutional pathologist coded 42% as having serous cystadenocarcinoma, 32% as having solid adenocarcinoma, 11% as having mucinous carcinoma, 8% as having endometrioid carcinoma, and 5% as having clear cell carcinoma. Tumor was coded as well-differentiated in 11%, moderately well-differentiated in 45%, and poorly differentiated in 32%; differentiation was not stated for 13%. Total abdominal hysterectomy and bilateral salpingo-oophorectomy had been accomplished in 63%. Prior chemotherapy had produced objective tumor regression in 29%, and tumor progression in 45%. Available information did not permit evaluation of response to prior chemotherapy in the remainder.

Disease was measurable on physical examination in 33 patients (chest film also showed measurable disease in one) and on ultrasonogram in one. Disease was "evaluable," but not measurable, on physical examination in the remaining four patients.

RESULTS

As shown in table 1, the toxicity of the initial schedule of 100 mg/m² of DDP followed by HXM was severe. The 31% rate of life-threatening thrombocytopenia (platelet count < 25,000/μl) combined with the 23% rate of severe thrombocytopenia (platelet count, 25,000–50,000/μl) was sufficient to make us modify the protocol after 13 patients were entered, so that all patients were subsequently given 75 mg/m² of DDP. As can be seen in table 1, hematologic toxicity was within generally acceptable limits at the lower dose of DDP. Three of 13 patients receiving the higher dose of DDP developed azotemia, with peak serum creatinine levels of 9.2 and 2.2 mg/dl in two and a peak BUN of 26 mg/dl in the third. The first of these patients was considered to have had life-threatening renal failure. She did survive this episode and lived a total of 6½ months from entry.

TABLE 1.—Toxicity

	HD-100 (13 patients)	HD-75 (25 patients)
Platelet count (cells/μl)		
< 25,000	31%	8%
25,000–50,000	23%	24%
Wbc count (cells/μl)		
< 1000	0	0
1000–2000	0	28%
Required transfusion	38%	29%
Serum creatinine (mg/dl) > 1.5	23%	8%

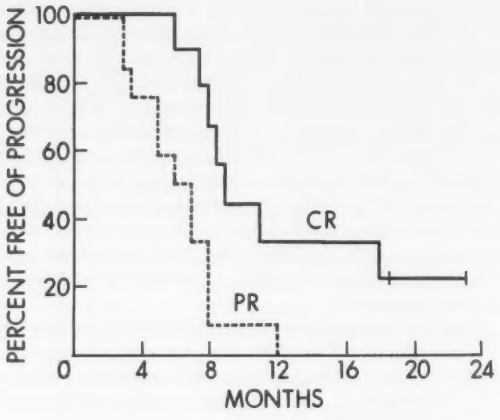

FIGURE 1.—Time to disease progression for partial and complete responders. Patients still in remission are indicated by vertical lines. Time of remission is measured from date of entry.

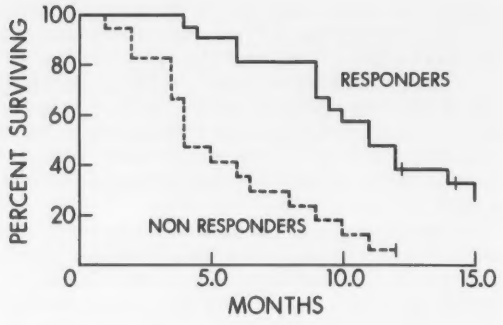

FIGURE 2.—Survival from date of entry as a function of response. Patients surviving at time of analysis are indicated by vertical lines.

In contrast to the high rate of azotemia seen at 100 mg/m² of DDP, only two patients (8%) who received 75 mg/m² developed transient increases of the serum creatinine to peak levels of 1.3 and 2.9 mg/dl. Renal function recovered promptly in both.

Hearing loss was noted in seven patients (18%) and was functionally significant in daily life for two of these. Thirteen patients (34%) developed peripheral neuropathy consisting largely of paresthesias, but six (16%) had functional limitation, with clumsiness, weakness, and poor balance in five and severe tremors in one.

Objective complete or partial remission of tumor was noted in seven of 13 patients receiving HD-100 (54%) and in 14 of 25 receiving HD-75 (56%). Nine of the 21 objective responses were complete by clinical and roentgenographic evaluation. Only one of four patients without measurable disease responded (partial remission, 3 months). The median duration of remission was 8 months (range, 3–23+). As shown in figure 1, the median duration of partial remission was 6.5 versus 9 months for clinical complete remission. As shown in figure 2, the median survival for patients with objective remissions was 10.8 months, significantly longer than that for those who did not respond (3.9 months; $P = 0.0006$, log-rank test).

Table 2 is an analysis of response rates by entry characteristics. None of the variables examined had significant influence on the rate of objective regression. In contrast, table 3 shows that the response rate for the 12 poorly differentiated tumors by Broders' criteria (nine of which were coded as solid adenocarcinomas), was only 25%, significantly lower than that for the remainder of the patients ($P = 0.016$, Fisher's exact test).

Table 4 is an analysis of prognostic factors for survival. Three factors were marginally significant for predicting prolonged survival: serous histology ($P = 0.025$, log-rank test) (15), Broders' grade 1, 2, or 3 ($P = 0.03$), and largest known tumor diameter at entry < 5 cm ($P = 0.03$). When these three factors were included in a multivariant analysis (Cox model [16]), the joint significance was $P = 0.07$. The decreased level of significance in the Cox model relates to the correlation between the factors and the relatively small sample size.

CONCLUSIONS

The results of this study establish the maximum tolerated dose of DDP as 75 mg/m² when combined with HXM in the treatment of patients with advanced ovarian cancer progressive in spite of alkylating-agent chemotherapy. Although DDP has been considered only minimally myelosuppressive in the past, thrombocytopenia proved to be the dose-limiting toxic effect in this patient population with extensive prior myelosuppressive treatment.

The present study served as a pilot trial for a later, ongoing, comparative, randomized, prospective clinical trial comparing two doses of DDP (37.5 or 75 mg/m²), each given with HXM. This comparative trial has been underway since February 1980 and should complete its patient accrual in 1982. The goal of this study is to determine whether the higher dose of DDP is necessary to improve response rate, quality of response, or duration of response.

The response rate noted in this study (55%) is

TABLE 2.—Response rates by entry characteristics*

Characteristic	Yes	No
Prior radiotherapy	50% (16)	59% (22)
Only 1 prior chemotherapy drug	64% (14)	50% (24)
PS 0 or 1	59% (27)	45% (11)
Age ≤ 56 yrs	58% (19)	53% (19)
Interval from diagnosis ≥ 14 mos	66% (18)	50% (20)
Weight loss < 5%	56% (25)	54% (13)
Coelomic involvement only	56% (27)	55% (11)
Bulk† (cm)		
≤ 5	64% (14)	48% (21)
> 10	56% (25)	50% (10)

*Values in parentheses = No. of patients.
†Largest diameter of known tumor at entry (unknown in 3 patients).

TABLE 3.—Response rates by histologic type and grade*

	Response rate (No. of patients)
Histologic type	
Serous cystadenocarcinoma*	75% (16)
Solid adenocarcinoma†	38% (13)
Mucinous	50% (4)
Endometroid	0 (3)
Clear cell	50% (2)
Tumor grade (Broders')	
Well-differentiated, grade 1	75% (4)
Moderately differentiated, grade 2 and 3	65% (17)
Poorly differentiated, grade 4	25% (12)
Unknown	80% (5)

*Based on evaluation of pathologist at institution in which patient was first evaluated; values in parentheses = No. of patients.
†9 of 13 patients with adenocarcinoma had poorly differentiated tumors.

equal to that previously noted with HAD (8). The results are considerably better than those previously reported with either HXM or DDP as single agents (1–6) and somewhat better than those reported for combinations of DDP with doxorubicin plus cyclophosphamide (17); DDP with doxorubicin, cyclophosphamide, and HXM (18); or DDP with doxorubicin, 5-FU, and HXM in similar patient populations (19). Since doxorubicin, 5-FU, and cyclophosphamide have response rates < 10% in patients with progressive disease receiving alkylating-agent therapy (7,10,11), it is not surprising that their use in combination therapy in this patient population increases toxicity without increasing response rate. In the present study, as in our previous study (8), we are unable to identify any prognostic factors for response except for histologic grade.

The high rate of peripheral neuropathy in the present trial is a matter of concern. The overall incidence of 34%, with 16% having functional limitation, is considerably higher than that observed in our prior trial with HXM plus 50 mg/m^2 of DDP (12% peripheral neuropathy without any instance of functional limitation) (8). The peripheral neuropathy is probably due in part to HXM, but the higher rate in the present study may be a result of a higher DDP dose. DDP administration alone has been reported to cause peripheral neuropathy in approximately 3% of patients (20). We have seen only one case of peripheral neuropathy in approximately 150 patients treated with DDP who did not receive concomitant vincristine or HXM (known neurotoxic agents) (21). Since a Southwest Oncology Group study of HXM in breast cancer suggested, but did not prove, that concomitant administration of pyridoxine (vitamin B_6) reduces the incidence and severity of peripheral neuropathy from HXM (22), one half of the patients in our randomized trial will receive prophylactic pyridoxine at a dose of 300 mg/day while they are receiving HXM and DDP.

Of 38 evaluable patients in the current trial, only two remain in clinical complete remission 18 and 23 months after entry. While it remains conceivable that one or both of these patients is cured, it is clear that reserving the administration of HXM and DDP until failure of alkylating-agent chemotherapy will not have substantial impact on the overall survival of women with advanced ovarian cancer. We have therefore advocated the use of these agents as part of initial chemotherapy, in which we have already shown them to substantially improve the rate of objective response as well as the quality of these responses (23,24).

TABLE 4.—Median survival in mos by entry characteristic*

	Yes	No
PS 0 or 1	8.5 (27)	7.3 (11)
Complete or partial remission	10.8 (21)	3.9 (17)
Serous histology	10.8 (16)	5.8 (22)
Broders' grade 1, 2, or 3†	9.8 (21)	5.5 (12)
Age ≤ 56 yrs	9.5 (19)	5.8 (19)
Bulk ≤ 5 cm‡	11.0 (14)	5.8 (21)
Weight loss < 5%	9.0 (25)	6.0 (13)
Interval from diagnosis > 12 mos	9.3 (19)	7.0 (19)
Prior TAH-BSO§	8.5 (17)	7.8 (21)
Coelomic involvement only	9.0 (27)	6.0 (11)
Prior single drug only	9.0 (14)	6.0 (24)

*Values in parentheses = No. of patients.
†Grade is unknown in 5 patients.
‡Largest diameter of known tumor at entry (unknown in 3 patients).
§Total abdominal hysterectomy-bilateral salpingo-oophorectomy.

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Chemotherapy Versus Chemoimmunotherapy With Levamisole or *Corynebacterium parvum* in Advanced Lung Cancer[1,2]

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SUMMARY

A total of 109 patients with advanced lung cancer, all cell types, were randomized between MACC chemotherapy only, consisting of methotrexate, doxorubicin (Adriamycin), cyclophosphamide, and lomustine (CCNU); MACC plus levamisole (LMS) orally; and MACC plus *Corynebacterium parvum* (CP) sc. Of these patients, 101 were evaluable, with no differences among the three treatment groups for overall response rate and survival time. Objective response rates and median survival times were 41% and 230 days for patients given MACC only, 39% and 257 days for those given MACC plus LMS, and 44% and 223 days for those given MACC plus CP, respectively. There was a significant increase in survival for patients with large cell anaplastic carcinoma receiving CP or LMS, particularly in the good-performance-status category. Pretreatment delayed cutaneous hypersensitivity to recall antigens in 50 patients had prognostic significance, but repeat tests after 2 months of treatment in 30 patients did not show different patterns of conversion among the three groups. There was no difference in hematologic toxicity among the three groups. With the possible exception of large cell anaplastic carcinoma, immunotherapy with LMS or CP as given in this trial does not appear to be therapeutically advantageous in advanced lung cancer.

[Cancer Treat Rep 66:1291–1297, 1982]

The results of chemotherapy for advanced lung cancer, particularly in cell types other than small cell anaplastic, have been disappointing. Despite combination chemotherapy, the overall 1-year survival generally remains < 10% (1). Only a few chemotherapeutic agents have shown some degree of activity. Following our results (2,3) with a four-drug combination, MACC, consisting of methotrexate, doxorubicin (Adriamycin), cyclophosphamide, and lomustine (CCNU), we decided to investigate the role of immunotherapy in addition to chemotherapy in advanced lung cancer. Immunotherapy is most effective in cases of minimal tumor burden. However, on the basis of previous trials and because of the lack of new effective chemotherapeutic agents, the present randomized trial was undertaken in patients with advanced lung cancer. Two immunotherapeutic agents were selected. When combined with chemotherapy, *Corynebacterium parvum* (CP) treatment has produced increased survival but not increased response rates in three randomized trials in advanced lung cancer (4–6). Levamisole (LMS), when used as an adjuvant after surgery for lung cancer, was effective in delaying recurrence and

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increasing survival, but only in patients weighing < 70 kg, allegedly because the dose of LMS was constant and not adjusted to weight or body surface in that trial (7). Clinical studies have suggested that these immunomodulators may restore delayed cutaneous hypersensitivity in cancer patients (4,8) and reduce hematologic toxicity from chemotherapy (4,9).

MATERIALS AND METHODS

Patient Population

A total of 109 consecutive patients with advanced, progressing, histologically proven bronchogenic carcinoma entered the study from March 1976 to March 1978. Cell types were categorized according to the World Health Organization's classification; all patients had advanced disease (stage III) (3). No patient should have received prior chemotherapy. Extent of disease and clinical workup have been defined previously (3). Performance status (PS) was evaluated according to the Cancer and Leukemia Group B scale: 0 = ambulatory without symptoms; 1 = ambulatory with symptoms; 2 = < 50% of the daytime in bed; 3 = > 50% of the daytime in bed; and 4 = bedridden.

Informed consent was obtained after the nature of the treatment had been fully explained. Patients were stratified within each of four major cell types (adenocarcinoma, squamous cell, large cell anaplastic, and small cell anaplastic) and were randomized among the three treatment groups. The first group received chemotherapy with the four-drug MACC combination, consisting of 30–40 mg/m² of methotrexate iv, 30–40 mg/m² of doxorubicin iv, 300–400 mg/m² of cyclophosphamide iv, and 30 mg/m² of lomustine orally (3). This regimen was repeated once every 3 weeks in the absence of residual toxicity. Peripheral blood cell counts were repeated every 10 days and the dose of chemotherapy was adjusted according to the nadir wbc and/or platelet counts. Doxorubicin was discontinued after a total dose of 550 mg/m². The second group received MACC and LMS (supplied in 50-mg tablets) at a dose of 2.5 mg/kg orally during 2 consecutive days every week. The third group received MACC and CP at a dose of 3.5 mg (0.5 ml) sc once a week, starting with chemotherapy. Each dose of CP was given at the root of one extremity by using a rotating schedule to minimize possible disability from local reaction. Criteria of response and duration of treatment have been defined previously (3).

Whenever a treatment delay ≥ 48 hours was possible, delayed cutaneous hypersensitivity (DCH) skin tests were performed intradermally before the first treatment with five recall antigens including 5 units of purified protein derivative (Parke-Davis, Detroit, MI), 0.1 ml of mumps antigen (Eli Lilly and Co, Indianapolis, IN), 0.1 ml of a 1:100 dilution of Dermatophytin "O" (Hollister-Stier Labs, Spokane, WA), 0.1 ml of a 1:30 dilution of Dermatophytin (Hollister-Stier Labs), 40 units of streptokinase, and 10 units of streptodornase (Lederle Laboratories, Pearl River, NY). A positive test was defined by an induration ≥ 5 mm at 48 hours.

Statistical Methods

The characteristics of patients randomized to the three groups were examined at the beginning of the study to determine whether the groups were comparable using methods of descriptive statistics, chi-square tests, and analysis of variance. Response rates overall and within strata defined by cell type were compared among treatment groups using chi-square tests. With 34 patients in each of any two treatment groups, an absolute difference of 35% from 30% in response rates would be detectable with a significance level of 10% (two-sided) and a power of 80%; a difference of 25% would be detectable with a power of 50%. Kaplan-Meier cumulative survival curves from date of first treatment (which was within days of randomization) were compared for the treatment groups using Breslow's extension of Gehan's generalized Wilcoxon test and the Mantel-Cox log-rank chi-square test (10). When results with both tests were similar, only log-rank values are given. Median survival times are shown for descriptive purposes only. Cox regression models for censored data were also used to further examine possible prognostic factors jointly in relation to treatment (11). All P values are given for two-tailed tests.

RESULTS

Of the 109 randomized patients, eight were unevaluable (four were lost to followup, two had prior chemotherapy with components of MACC, one had an inadequate dose of chemotherapy, and one had no measurable disease). The present analysis is therefore restricted to the remaining 101 evaluable patients, five of whom are still alive. Patient characteristics were comparable in the three groups (table 1). There was no difference in age distribution among the three arms: mean age of 57.3 years for MACC alone, 59.9 for MACC plus LMS, and 58.2 for MACC plus CP.

Response to Therapy

Response rates are shown in table 2. No significant differences were noted among the three groups

TABLE 1.—Patient characteristics

Characteristic	No. of patients (%) treated with—		
	MACC only	MACC + LMS	MACC + CP
Evaluable patients	34 (100)	33 (100)	34 (100)
Sex			
Male	24 (71)	23 (70)	23 (68)
Female	10 (29)	10 (30)	11 (32)
PS			
0	9 (27)	10 (30)	6 (18)
1	11 (32)	15 (46)	16 (47)
2	9 (27)	7 (21)	7 (20)
3	4 (12)	1 (3)	5 (15)
4	1 —	0 —	0 —
Prior treatment			
Surgery	5 (15)	2 (6)	4 (12)
Radiotherapy (lung)	9 (26)	10 (30)	10 (29)
Extent of disease			
Limited	7 (21)	7 (21)	8 (24)
Extensive	27 (79)	26 (79)	26 (76)
Metastases			
Bone	15 (44)	16 (48)	11 (32)
Pleura	3 (9)	4 (12)	8 (24)
Lung	7 (21)	1 (3)	4 (12)
Liver	3 (9)	4 (12)	5 (15)
Brain	1 (3)	3 (9)	1 (3)
Soft tissue	3 (9)	1 (3)	1 (3)

overall nor within each cell type. The duration of therapy until disease progression ranged from a mean of 160 days for patients given MACC plus LMS to 198 days for patients given MACC plus CP. No major differences were seen when response rates among the three groups were compared within strata defined by patient characteristics (age, sex, extent of disease, cell type, and PS).

Survival

Overall survival (fig 1) was nearly identical among the groups for all evaluable patients (log-rank $\chi^2_2 = 0.3$; $P = 0.9$). These results were unchanged when the eight nonevaluable patients were included. The observed 1-year cumulative survival rate was 32% (SE = 8%) in each of the three treat-

TABLE 2.—% objective response by cell type*

Cell type	% response							
	MACC only		MACC + LMS		MACC + CP		Total	
	CR	PR	CR	PR	CR	PR	CR	PR
Adenocarcinoma	11	33	8	33	9	9	9	25
	(N = 9)		(N = 12)		(N = 11)		(N = 32)	
Squamous cell	0	20	0	33	20	30	8	27
	(N = 10)		(N = 6)		(N = 10)		(N = 26)	
Large cell anaplastic	0	33	0	50	12	50	5	45
	(N = 6)		(N = 6)		(N = 8)		(N = 20)	
Small cell anaplastic	44	22	33	0	60	0	43	9
	(N = 9)		(N = 9)		(N = 5)		(N = 23)	
Total	15	26	12	27	20	24	16	26
	(N = 34)		(N = 33)		(N = 34)		(N = 101)	

*Objective response includes complete response (CR)—the disappearance of all clinical, radiologic, and laboratory evidence of tumor, and partial response (PR)—a > 50% decrease in the sum of the product(s) of perpendicular diameters of measurable lesion(s) without the appearance of new lesion(s) elsewhere.

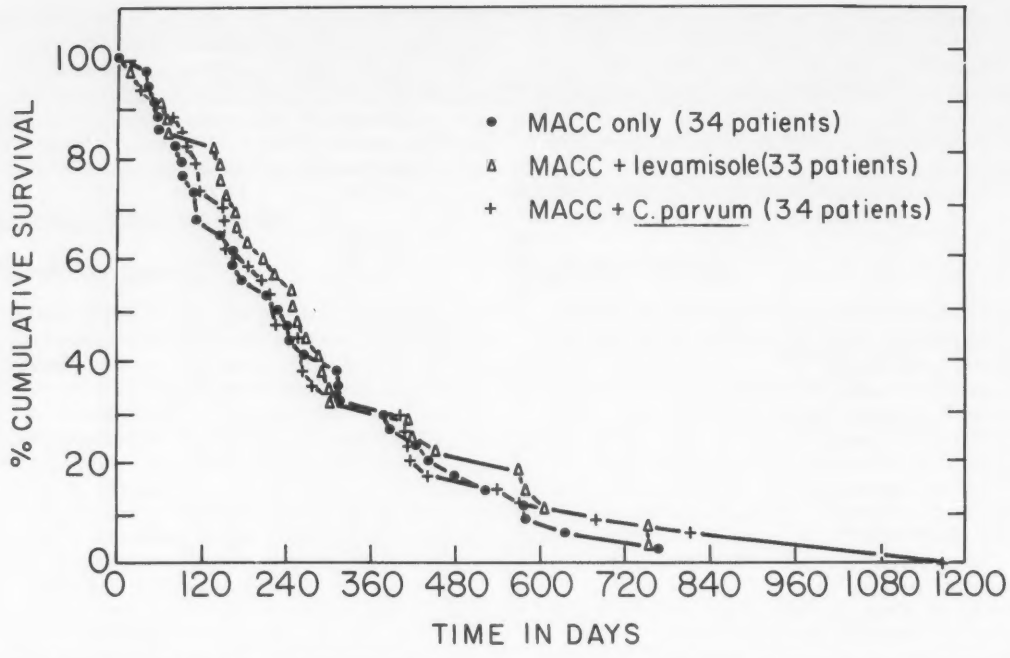

FIGURE 1.—Survival in days from 1st treatment of all 101 evaluable patients with advanced lung cancer, all cell types combined, randomized to receive MACC chemotherapy only, MACC + levamisole, or MACC + *C. parvum*. Survival curves are nearly identical among the 3 groups.

ment groups. Eight patients survived > 2 years (three with small cell, three with large cell, one with adenocarcinoma, and one with squamous cell). They were distributed among the three treatment groups and included five complete responders, two partial responders, and one patient with stable disease. Table 3 shows median survival times for subgroups of patients. Regardless of treatment group, the median survival of all 42 objective responders (419 days) was significantly longer than the median survival of all 59 nonresponders (155 days) (log-rank χ^2_1 = 33.1; $P < 0.005$). This difference remained significant within major PS categories (0 and 1 or ⩾ 2). For patients with a poor PS (⩾ 2), there was a suggestion of lengthened survival with immunotherapy (log-rank χ^2_2 = 6.7; P = 0.04).

There were no significant differences in survival among the three arms when sex, response to therapy, or extent of disease was considered.

Further multivariate analyses of survival using Cox regression models were carried out to determine whether any factors jointly affected prognosis in relation to treatment. When treatment, sex, age, PS, cell type (small cell vs non-small cell), and extent of disease (limited vs extensive) were included in the models, the observed mortality was not accounted for by these variables jointly (χ^2_8 = 6.65; $0.50 > P > 0.25$). Of these variables, only PS appeared to have some prognostic impact (coefficient/SE = 1.77; $0.05 < P < 0.10$) when adjusted for other variables.

Comparison of treatment groups within cell types indicated that the median survival for the few patients with large cell anaplastic carcinoma was significantly longer with MACC plus LMS or MACC plus CP compared to MACC only (log-rank χ^2_2 = 14.6; P = 0.001). This difference persisted when only patients with PS 0 or 1 were considered. No difference in survival among the three arms was seen in patients with small cell anaplastic carcinoma who responded, although their number was small.

Pretreatment DCH skin tests were performed on 50 patients distributed without bias among all treatment groups and cell types. Regardless of treatment group, median survival was 91 days for 12 patients with all skin tests negative and 287 days for 38 patients with at least one positive skin test (log-rank χ^2_2 = 16.1; P = 0.0001). There was no significant difference in survival with regard to skin tests among the three treatment groups, nor for

TABLE 3.—Median survival (days) by treatment group

	MACC only	MACC + LMS	MACC + CP
All patients	230	257	223
Sex			
All males	178	291	205
All females	242	171	404
Response to treatment			
Objective responders	426	419	407
Nonresponders	114	171	153
Extent of disease			
Limited	312	287	261
Extensive	230	251	155
PS			
0 or 1	478	250	227
⩾ 2	109	300	220
Cell type			
All except small cell	178	291	223
Adenocarcinoma	387	171	205
Squamous cell	166	291	189
Large cell anaplastic	114	419	407
Small cell anaplastic	316	208	257

one versus two or more positive tests. No apparent association was found between the number of positive pretreatment DCH skin tests and PS categories.

Toxicity

Toxicity was primarily hematologic, without significant differences between the three arms (table 4). The mean number of courses of chemotherapy needed to reach nadir wbc counts was 4.4 for MACC only, 4.5 for MACC plus LMS, and 4.7 for MACC plus CP. Similar figures for nadir platelet counts were 5.7, 4.4, and 3.2, respectively. None of these differences was significant. Mucositis occurred in 16 patients (seven given MACC only, three given MACC plus LMS, and six given MACC plus CP). Fever and infection during leukopenia were seen in seven patients. Cardiac toxicity included atrial flutter, atrial premature contractions, and decreased QRS volt-

TABLE 4.—Drug dose and hematologic toxicity

	MACC only	MACC + LMS	MACC + CP
No. of cycles of MACC			
Mean ± SE	8.0 ± 1.08	7.5 ± 1.01	8.0 ± 1.08
Range	1–24	1–23	1–25
Average drug dose (mg/m²)/patient/course			
Methotrexate	29.7	29.8	31.4
Doxorubicin	32.2	32.2	32.2
Cyclophosphamide	352.9	344.0	350.7
Lomustine	26.6	25.6	27.6
Wbc counts (cells/mm³)			
Nadir (mean ± SE) for all patients	3990 ± 740	3470 ± 370	4650 ± 710
< 3000 cells/mm³			
No. of patients	15	16	14
Median nadir	1100	1750	1750
Range	225–2900	400–2700	400–2700
Platelet counts (cells/mm³)			
Nadir (mean ± SE) × 10^3 for all patients	163 ± 21	148 ± 15	197 ± 24
< 100,000 cells/mm³			
No. of patients	5	7	8
Median nadir × 10^3	55	69	70
Range × 10^3	20–92	10–90	10–98

age > 30% (one patient each). Only one patient developed congestive heart failure after receiving the full dose of doxorubicin. However, this patient responded to digoxin and diuretics. Hemorrhagic cystitis occurred only once. There were no significant hepatic or renal toxic effects. There was one treatment-related death from pneumonitis and fulminant septic shock during leukopenia in a patient receiving MACC plus LMS.

Immunotherapy was well-tolerated. Injections of CP were minimally painful, not requiring the use of local anesthetics, and resulted in slight to moderate local induration. A 25-mm ulceration occurred in only one patient. Two patients developed fever of 100°F–102°F after CP, and it was discontinued in one of them. Nine patients received CP every 3 weeks only, together with chemotherapy, because of the inconvenience of commuting weekly. Their response rate and survival were not different from patients receiving weekly CP. LMS was also well-tolerated. The most common side effect was a sensation of metallic taste (four patients). Nausea and vomiting occurred in one patient, and a maculopapular facial rash was observed in another. Agranulocytosis was seen in two patients. It was difficult to ascribe it to LMS, however, since all patients received chemotherapy concomitantly and since overall hematologic toxicity was not different among the three arms. One of these patients developed a leukopenia of 1500 cells/mm^3, with 2% neutrophils, 2% eosinophils, 23% monocytes, and 73% lymphocytes after the fifth cycle of MACC; there was also a mild thrombocytopenia of 122,000 cells/mm^3. Blood cell counts returned to normal within 5 weeks and no further hematologic toxicity occurred with the same dose of chemotherapy without LMS. The other patient developed a leukopenia of 1500 cells/mm^3 with 0% neutrophils, 3% eosinophils, 1% monocytes, and 96% lymphocytes (after the 11th cycle of MACC); there was also a thrombocytopenia of 80,000 cells/mm^3. Blood cell counts returned to normal within 3 weeks. No further hematologic toxicity was seen with the same dose of chemotherapy without LMS. This patient was rechallenged with LMS 2 months later without hematologic toxicity.

DISCUSSION

The present study extends our earlier reports of MACC chemotherapy in advanced lung cancer (2,3). The overall median survival of 33 weeks for patients receiving MACC is very close to the 36 weeks previously reported in a pilot trial of 83 patients similarly treated (3). A recent trial of MACC by the Eastern Cooperative Oncology Group showed a low response rate and prohibitive toxic reactions in patients with squamous cell carcinoma and adenocarcinoma of the lung (12). Several factors that may have contributed to these discrepancies have been discussed (13). Cohen et al (14) reported a 23% response rate, with prolongation of survival without prohibitive toxic effects in 72 patients with advanced lung cancer treated with MACC at the lower dose range. Corkery et al (15) obtained a 22% response rate (35% in limited disease) in 40 patients with lung cancer (cell types other than small cell) treated with a modified MACC regimen given on Days 1 and 8 every 6 weeks. In an ongoing trial of the North Central Cancer Treatment Group, patients with advanced adenocarcinoma and large cell anaplastic carcinoma of the lung are randomized to receive chemotherapy with MACC or cyclophosphamide, doxorubicin, and cisplatin. Response rates are presently similar (35% and 33%, respectively), but gastrointestinal toxicity was more acceptable with MACC (16). In a randomized trial of 39 patients with advanced lung cancer (cell types other than small cell), median survival was 34.5 weeks for MACC compared to 8.5 weeks for a placebo (17).

The addition of LMS or CP as given in this randomized trial did not increase overall response rates or survival. There was no bone marrow protection from drug toxicity by either CP or LMS and no demonstrable effect on DCH skin test when compared to chemotherapy alone. Similarly, other investigators using various routes, doses, and timing of CP in relation to chemotherapy have failed to show an improvement in survival (18–21), drug-induced hematologic toxicity (18,20), or DCH skin tests (18) in patients with advanced lung cancer. The activity of LMS as an adjuvant to surgery in lung cancer has not been confirmed in two trials (22,23), and its effect on DCH skin tests has been questioned (24).

Results of multiple significance tests carried out within subgroups must be interpreted with caution. Analysis by cell type in the present trial showed a significant benefit for immunotherapy in patients with large cell anaplastic lung cancer. This was particularly true in patents with a good PS (0 or 1). On the other hand, for all lung cancer types except large cell anaplastic, immunotherapy did not appear to be therapeutically advantageous. The number of patients with large cell anaplastic lung cancer remains small and confirmation is required. These results suggest that there may be different effects of immunotherapy on lung cancer according to cell type and cast serious doubts on studies of "composite lung cancer" cases. Just as small cell anaplastic cancer has been removed from lumped considerations because of differential chemotherapeutic response, so too large cell anaplastic carcinoma may appro-

priately be removed for future studies from the group of "non-small cell" type because of differential host response.

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Treatment of Advanced Malignant Melanoma With Vinblastine, Bleomycin, and Cisplatin[1]

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SUMMARY

Thirty-two consecutive patients with metastatic malignant melanoma were treated with vinblastine, bleomycin, and cisplatin (VBD) chemotherapy. There were 29 evaluable patients; complete remission was observed in three and partial remission in five. No patient with visceral metastasis showed objective response. The 10-month survival rate of responders was significantly higher than that of nonresponders (57.1% vs 18.2%, $P = 0.01$); however, present results do not confirm that VBD is superior to other drug combinations for malignant melanoma.

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Dacarbazine (DTIC) is still the treatment of first choice for many patients with metastatic malignant melanoma and, irrespective of the dose schedule (1), provides objective response in 18%–25% of patients, particularly at the level of soft tissue involvement (2,3). DTIC has also been utilized in several drug combinations, with (4,5) and without (6,7) immunostimulants, but the response rate was not significantly improved. Therefore, it appears worthwhile to study new drugs (8,9) and new combinations to verify their efficacy in malignant melanoma.

In 1980, Nathanson et al (10) reported a 71% objective response rate in 14 evaluable patients, utilizing a new treatment schedule: vinblastine, bleomycin, and cisplatin (VBD). This combination has also shown objective response in patients with visceral disease.

At the Istituto Nazionale Tumori of Milan we have tested VBD chemotherapy in metastatic melanoma, and the experience achieved with this drug regimen is the subject of the present report.

PATIENTS AND METHODS

From August 1980 to January 1981, 32 consecutive patients with metastatic malignant melanoma were treated with VBD. The main patient characteristics are listed in table 1. Twenty-five of 32 patients presented with multiple sites of involvement.

TABLE 1.—Patient characteristics

Characteristic	No. of patients	(%)
Total entered	32	
Total evaluable	29	(90.6)
Males/females	16/13	
Median age in yrs (range): 52 (21–65)		
Previous chemotherapy	18	(62)
Disease extent		
Soft tissues	19	(65.5)
Viscera ± soft tissues	10	(34.5)
Dose of vinblastine		
6 mg/m²	12	(41.3)
4 mg/m²	17	(58.7)
Total not evaluable	3	(9.4)
Early death	1	
Inadequate treatment*	2	

*< 1 full cycle.

Patients aged > 70 years or with important chronic nonneoplastic disease or brain metastases were considered ineligible for this trial. Before starting therapy, it was required that patients have measurable parameters for drug response, a life expectancy ≥ 6 weeks, and a Karnofsky performance status ≥ 40. Eighteen patients (62%) were previously treated with chemotherapy: 13 with DTIC, and five with other drugs. No patient had had prior therapy with vinblastine, bleomycin, or cisplatin.

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TABLE 2.—Response to VBD in 29 evaluable patients

	No. of patients			
	Evaluable	CR + PR	CR	MR
Total	29	8 (27.6%)	3	10
Males	16	3	1	6
Females	13	5	2	4
Soft tissues	19	8 (42.1%)	3	4
Skin	9	4	—	4
Nodes	10	4	3	2
Viscera	10	0	0	6

Treatment was performed in the hospital ward. The drug schedule was as follows. Vinblastine (6 mg/m^2) was injected iv on Days 1 and 2, bleomycin (15 mg/m^2) was administered by 24-hour iv infusion from Day 1 to Day 5, and cisplatin[3] (50 mg/m^2) was given iv by 1-hour infusion on Day 5. In an attempt to minimize myelotoxicity, the dose of vinblastine was reduced to 4 mg/m^2 starting with the 16th patient. Cisplatin was administered in 250 ml of 5% glucose in water, followed by 1000 ml of 5% glucose solution and 500 ml of 0.9% saline solution with 20 mEq/liter of KCl. Following hydration, patients were advised to drink 1 liter of fluid during the daytime. Patients were discharged on the following day. The regimen was repeated every 3 weeks. Cycles were delayed 1 week in the presence of persisting myelosuppression. After this delay, if the wbc count was between 2500 and 3600/mm^3 and/or the platelet count was between 75,000 and 100,000/mm^3, 50% of the planned doses of cisplatin and vinblastine were given.

An adequate therapeutic trial was defined as one full cycle of therapy. Chemotherapy was continued until disease progression occurred, and in patients who achieved complete response, two additional treatment cycles were administered. The maximum number of cycles administered was five. When VBD therapy failed, patients were given DTIC if they had not been previously treated with this drug.

Pretreatment hemogram, liver chemistry, serum calcium, renal function tests, and appropriate roentgenograms were obtained in all patients. Patients were evaluated before each treatment cycle with physical examination, repeated blood studies, X-rays, and scans as needed. Complete response (CR) was defined as the complete disappearance of all neoplastic lesions for at least 1 month. Partial response (PR) was defined as a $> 50\%$ decrease in the product of the two largest perpendicular diameters in all measurable lesions for a minimum of 1 month. A $< 50\%$ decrease in the product of the two largest perpendicular diameters of all measurable lesions was defined as minor response (MR). A $\geq 25\%$ increase in initial lesions was considered as disease progression, regardless of concomitant regression in other tumor sites or occurrence of new lesions.

[3]Provided by Bristol-Myers Co, International Division, New York, NY, and Farmitalia-Carlo Erba, Milan, Italy.

RESULTS

Of the 32 patients admitted to the study, 29 are included in the analyses for response and survival. Three patients were not evaluable: one because of early death unrelated to treatment and two because of inadequate treatment (less than one full cycle). None of the patients refused to complete treatment. Two patients received only one cycle, ten received two cycles, and eight received three cycles, because the disease was rapidly progressing. Of 86 cycles administered, 14 (16.2%) were given without cisplatin because of leukopenia and/or thrombocytopenia on Day 5. In particular, this occurred in five of 30 cycles given to patients with visceral disease. As shown in table 2, CR occurred in three (10.4%) of 29 evaluable patients, PR in five (17.2%), and MR in ten (34.4%). Of patients achieving CR or PR (27.6%), there was no substantial difference between the percent who had had no previous treatment (10.3%) and the percent who had received prior chemotherapy (17.3%). The response rate was also unrelated to the dose of vinblastine. The majority of responses were documented after the first two treatment cycles.

All eight patients achieving CR or PR had metastatic disease only in the soft tissues. MR was observed in six patients with visceral involvement (lung, three; liver, three). The median duration of CR plus PR was 3 months (range, 1–10). Two patients who achieved CR are still alive, and one is still disease free after 10 months. With the exception of one patient, the site of relapse in responders occurred in previously involved sites.

Figure 1 shows that the survival rate of respond-

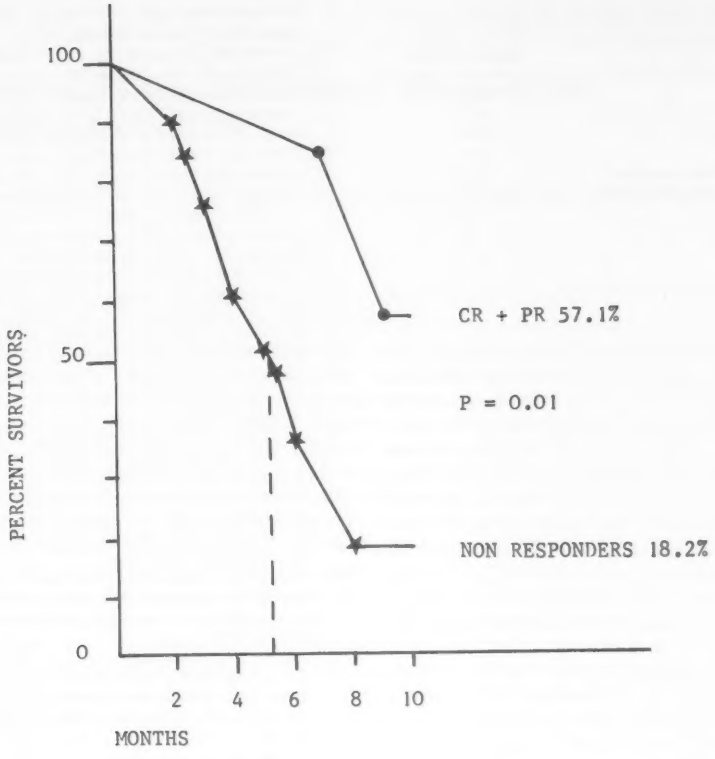

FIGURE 1.—Survival of responders.

ers was significantly higher than that of nonresponders (57.1% vs 18.2%, $P = 0.01$). The 10-month analysis from start of chemotherapy indicated that survival rate was significantly better for patients with soft tisue involvement than for patients with visceral deposits (39.1% vs 14.9%, $P < 0.02$). On the contrary, there was no significant difference in survival rates between patients previously treated or untreated with chemotherapy (25.7% vs 49%, $P = 0.39$) or between females and males (42.1% vs 27.3%, $P = 0.55$).

No drug-related deaths were observed. The incidence of side effects is shown in table 3. Fever, nausea, and severe vomiting were the most frequent toxic effects, while myelosuppression was the main treatment-limiting factor. Eleven patients had wbc counts $< 1000/mm^3$, 12 had platelet counts $< 60,000/mm^3$, and the nadir for both counts after each cycle occurred from Day 4 to Day 7. The decrease in wbc count appeared to be a cumulative toxic effect but the decrease in platelet count did not. None of the patients had renal abnormalities, and serum creatinine and blood urea nitrogen, in particular, always ranged within normal values. The incidence of side effects was about the same in previously untreated and treated patients.

CONCLUSION

Our prospective study failed to confirm the original data of Nathanson et al (10). In particular, no patient with visceral metastasis achieved objective response. VBD was as effective as other drug combinations recently tested in metastatic malignant melanoma (11–13), and considering the known limitation of cisplatin in terms of cumulative toxicity, VBD does not appear to be indicated for general use. More recently, Nathanson et al (14) have expanded their experience with VBD and reported an objective response in 43% of all patients. There were three CRs in patients with visceral disease and none in patients with soft tissue involvement. On the con-

TABLE 3.—Incidence of side effects in 29 evaluable patients

Side effect	Incidence (%)
Stomatitis	10.3
Alopecia	65.5
Nausea and vomiting	89.6
Fever	100
Leukocyte count < 1000 cells/mm^3	34.4
Platelet count < 60,000 cells/mm^3	41.3
Adynamic ileus	17.2
Skin changes	3.4
Bronchopneumonia	3.4
Herpes zoster	3.4

trary, we have observed three CRs in patients with soft tissue metastases and only MR in six of ten patients with visceral deposits. The observed differences in total response rates between our series and that of Nathanson et al could be due, in part, to the more aggressive schedule used by the American group: the dose of cisplatin was not modified in leukopenic patients on Day 5 (14). The difference in the aggressiveness of treatment was also reflected in the incidence of side effects (eg, abnormal pulmonary function tests and mild renal impairment), which were observed in the series of Nathanson et al and not in our own.

Our findings reemphasize that positive results utilizing a limited series of patients can often be misleading, as observed by Abele et al (15). In fact, with the combination of CCNU (lomustine), vincristine, and bleomycin, the Belgian group had initially observed an unusually high rate of 40%, which subsequently decreased to 25% when the total number of patients entered in the study became large.

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Phase I Study of a 5-Day Schedule of Mitoxantrone (Dihydroxyanthracenedione)[1,2]

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SUMMARY

Mitoxantrone (1,4-dihydroxy-5,8-bis[[2-[(2-hydroxyethyl)amino]ethyl]-amino]-9,10-anthracenedione dihydrochloride) in animal studies appears to have a mechanism of action and broad antitumor spectrum similar to the anthracyclines and in preliminary animal studies is without cardiotoxicity. To determine the maximally tolerated dose, mitoxantrone was given to 25 patients with various advanced solid tumors. Sixteen patients had received prior doxorubicin and/or lomustine (CCNU). Myelosuppression was the major toxic effect. All 13 patients who received mitoxantrone doses ≥ 2.73 mg/m² × 5 days experienced moderate (2000–3000 cells/mm³) or severe (< 2000 cells/mm³) leukopenia. Thrombocytopenia was also encountered in three of these 13 patients (severe in one, < 50,000 cells/mm³). At dose levels < 2.73 mg/m² × 5 days, myelosuppression was seen in four of 12 patients (three with mild leukopenia and one with mild thrombocytopenia). The blood cell count nadir occurred at 10 days and full recovery occurred by Day 21. Minor clinically insignificant ECG changes occurred in five patients. Minor and transient antitumor effects were seen in five patients. The maximum tolerated dose of mitoxantrone over a 5-day course is 2.73 mg/m², with leukopenia being the limiting toxic effect.

[Cancer Treat Rep 66:1303–1306, 1982]

Mitoxantrone (fig 1) belongs to the synthetic class known as the anthraquinones. Like doxorubicin, mitoxantrone binds to DNA, inhibiting nucleic acid synthesis (1,2).

Mitoxantrone shows activity in a wide range of experimental animal tumors. It is highly active against B16 melanoma, colon tumor 26, and P388 and L1210 leukemias. It showed moderate activity against P388/Adria and was ineffective when tested against Lewis lung carcinoma and Ridgway osteogenic sarcoma (3–5).

In contrast to the anthracyclines, preclinical studies have not demonstrated cardiotoxicity. In monkeys and dogs receiving mitoxantrone, no abnormalities were noted on auscultation, ECG, or blood pressure monitoring. Animals that were killed showed no cardiac pathology.[5] Ante- and post-mortem toxicity in animals has largely been confined to the gastrointestinal system and bone marrow. Because of this wide spectrum of antitumor activity and the apparent lack of cardiotoxicity, this anthracenedione appears to be an attractive candidate for clinical trials.

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[2]Supported by the Medical Research Division, American Cyanamid Co, Lederle Laboratories, Pearl River, NY, and presented in part at the 1981 meeting of the American Society of Clinical Oncology.

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[4]We thank Mrs. Judy Weigel for her help in preparing this manuscript.

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[5]Animal Studies of Cardiotoxicity With Mitoxantrone. Unpublished data on file in the Toxicology Research Section, Lederle Laboratories Division, American Cyanamid Co, Pearl River, NY.

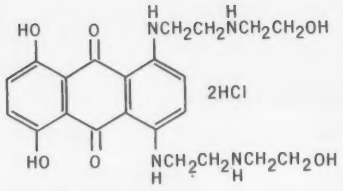

FIGURE 1.—Structure of mitoxantrone.

In a previous report, Von Hoff et al (6) treated 25 patients with a single iv dose every 4 weeks in a range of 1.2–14 mg/m² (6). We conducted a phase I clinical study of mitoxantrone using a schedule of daily doses for 5 consecutive days. The starting dose of 0.34 mg/m² was determined by taking two thirds of the "toxic dose low" in dog studies (0.515 mg/m²/day for 5 days). The goals of this study were to establish the maximum tolerated dose of mitoxantrone administered on a 5-day schedule and to determine the clinically significant toxic effects of this agent. The maximum tolerated dose was defined as that dose which would consistently produce a grade 3 (or greater) toxicity of a clinically serious nature in most patients.

MATERIALS AND METHODS

Patient Selection

Mitoxantrone was given to 25 patients (median age, 56 yrs; range, 39–73) with a variety of incurable solid tumors (table 1). Nine patients had received prior radiotherapy and 16 had received chemotherapy (ten received doxorubicin, six received cyclophosphamide, and six received lomustine [CCNU]). Eight patients had received no prior chemotherapy since it was felt that there was no effective standard chemotherapy for their extent and types of tumor.

On initiation of mitoxantrone, all patients had a Karnofsky performance status ≥ 60% and had an expected survival ≥ 2 months. No patient had received cytotoxic chemotherapy within the preceding month. None had a history of cardiac disease or was receiving antiarrhythmic medication. All patients had a wbc count > 4000/mm³ and a platelet count > 150,000/mm³. Two of 25 patients had hemoglobins < 10 g (one, 8 g and another, 9 g).

Written informed consent was obtained from all patients.

Treatment Schedule

Mitoxantrone was supplied by the Medical Research Division of the American Cyanamid Company as 0.5 mg/ml in 5- and 10-ml ampules. The drug was infused over 10–15 minutes in 250 ml of 5% dextrose in water on a daily × 5-day schedule monthly. The daily × 5-day dose level and number of patients at each level were as follows: 0.34 mg/m², four patients; 0.68 mg/m², three patients; 0.86 mg/m², two patients; 1.36 mg/m², three patients; 2.73 mg/m², five patients; 3.40 mg/m², six patients; and 4.00 mg/m², two patients. Patients were retreated at the same dose level every 4 weeks if they had experienced no toxicity, or at a lower dose level or not at all if severe toxicity had occurred with the first cycle.

Study Parameters

The following weekly tests were conducted with the first course of treatment: cbc with differential, platelet count, reticulocyte count, liver and renal function tests, electrolytes, glucose, calcium, phosphorus, CPK with MB fraction, ECG, and urinalysis. With subsequent courses, the cbc with differential and platelet count were repeated while the remainder of the above tests were performed monthly. Chest X-ray was obtained before each course. Patients were considered evaluable for toxicity if they received one dose of mitoxantrone. Standard response criteria were used to evaluate antitumor drug effect.

TABLE 1.—Types of solid tumors in 25 patients

Tumor type	No. of patients
Colorectal adenocarcinoma	11
Lung adenocarcinoma	4
Breast adenocarcinoma	2
Lung and larynx squamous cell carcinoma	1
Gastric adenocarcinoma	1
Melanoma	1
Lymphoma	1
Hypernephroma and perineal leiomyosarcoma	1
Hepatoma	1
Pelvic spindle cell sarcoma	1
Testicular embryonal carcinoma	1
Total	25

RESULTS

Over a 10-month period (concluded October 1980) 25 patients received a total of 54 courses of mitoxantrone. Twenty-three of the 25 patients are fully evaluable, having received at least one full course (daily single dose for 5 consecutive days). One patient at the 4.0-mg/m² dose level refused further therapy after receiving the drug for only 3 days but is considered evaluable for toxicity. Another patient received one full course of therapy but was only partially evaluable because of inadequate followup laboratory studies. Eight patients received single courses, ten had two courses, and the remainder had a range of three to six courses. None received concomitant antineoplastic medication.

Toxicity

Myelosuppression, especially leukopenia, was the major dose-limiting toxic reaction (table 2). All 13 patients receiving mitoxantrone at ≥ 2.73 mg/m² experienced moderate (2000–3000 cells/mm³) or severe (< 2000 cells/mm³) leukopenia and granulocytopenia. At dose levels < 2.73 mg/m², three patients experienced mild (3000–4500 cells/mm³) leukopenia. One patient with extensive liver metastases developed severe leukopenia (< 1000 cells/mm³) and thrombocytopenia (< 50,000 cells/mm³) at 0.68 mg/m²/day × 5 days.

Leukopenia, when it occurred, was seen with the initial course of mitoxantrone and recurred with subsequent courses at the same dose level. There did not appear to be any progressive decrease in the wbc count with repeated courses of therapy. Leukopenia appeared to be more prevalent in patients with extensive liver metastases. Three of four such patients experienced profound leukopenia (< 1000 cells/mm³). The 5-day dose levels of these three patients were 0.68, 2.73, and 3.4 mg/m².

Thrombocytopenia was encountered in three of 13 patients receiving ≥ 2.73 mg/m² (table 3). One patient with metastatic melanoma involving the liver who was treated with a dose of 3.4 mg/m² experienced profound leukopenia (wbc count, 200/mm³) and thrombocytopenia (platelet count, 29,000/mm³). The only possible drug-related death occurred in this patient, who had cerebral metastases and developed intracranial hemorrhage on Day 15 while still thrombocytopenic. Mitoxantrone-related myelosuppression generally reached a nadir at Day 10, with full recovery by Day 21. A mild decrease in

TABLE 2.—Wbc toxicity*

Dose (mg/m² daily × 5 days)	No. of patients	No. of courses	No. of courses by wbc count nadir, in cells/mm³ (grade)				
			> 4500 (0)	3000–4500 (1)	2000–3000 (2)	1000–2000 (3)	< 1000 (4)
0.34	4	6	6	0	0	0	0
0.68	3	3	1	1	0	0	1
0.86	2	4	3	1	0	0	0
1.36	3	16	8	4	1	3	0
2.73	5	10	2	1	1	2	4
3.40	6	12	3	1	1	3	4
4.00	2	3	1	0	1	0	1
Totals	25	54	24	8	4	8	10

*Mean nadir neutrophil count in patients with leukopenia was 412 cells/mm³ (range, 16–1240).

TABLE 3.—Platelet toxicity

Dose (mg/m² daily × 5 days)	No. of patients	No. of courses	No. of courses by platelet count nadir, in cells/mm³ (grade)				
			> 130,000 (0)	90,000–130,000 (1)	50,000–90,000 (2)	25,000–50,000 (3)	< 25,000 (4)
0.34	4	6	6	0	0	0	0
0.68	3	3	2	0	0	1	0
0.86	2	4	3	1	0	0	0
1.36	3	16	15	1	0	0	0
2.73	5	10	5	3	1	0	1
3.40	6	12	11	0	0	1	0
4.00	2	3	3	0	0	0	0
Totals	25	54	45	5	1	2	1

hemoglobin (1–2 g) was seen in 13 patients; however, this was probably a result of tumor progression rather than drug toxicity.

Nonhematologic Toxicity

Minor, clinically insignificant ECG changes were seen in five of 25 patients. These changes may have been related to mitoxantrone. Three of the five patients developed new, nonspecific ST-T wave changes, with two patients having such changes after the first cycle of mitoxantrone. A fourth patient developed new premature ventricular contractions. The premature ventricular contractions were noted 3 weeks after the second course of mitoxantrone and did not recur. A fifth patient experienced sinus tachycardia. Two of the above patients showing ST-T changes had received prior doxorubicin (one, a total of 300 mg/m^2). Radionuclide cardiac scanning was not performed in this study. There were no significant changes in serum CPK levels.

Other drug-related toxic reactions included mild nausea and vomiting in four patients and slight hair loss in three. No patient developed renal or liver dysfunction as a result of mitoxantrone therapy. There were no cases of phlebitis or toxicity to the skin or mucous membranes. Extravasation of small amounts of drug occurred in three patients but this produced no local tissue damage.

Antitumor Effects

In this phase I study involving 25 patients we observed modest objective antitumor effects only at dose levels of 3.4 mg/m^2. Four of eight patients receiving a 5-day course $\geq$ 3.4 mg/m^2/day showed an antitumor effect. There were two minor partial responses (< 50% shrinkage) and two disease stabilizations (one lung adenocarcinoma for 4 months and one colon carcinoma for 5 months). A patient with stage IV progressive, diffuse, poorly differentiated lymphocytic lymphoma showed a gratifying (but < 50%) tumor reduction in lymph node size after the first cycle of mitoxantrone. Unfortunately, he failed to return for further therapy. Another patient with pelvic spindle cell sarcoma had a minor partial regression lasting 3 months. Both of these patients had disease progression after prior extensive chemotherapy including doxorubicin. A final patient with epidermoid lung carcinoma, receiving 1.36 mg/m^2 over 5 days, showed disease stabilization of his lesion for 8 months.

DISCUSSION

The main goal of this study was to establish the maximum tolerated dose as defined above using the 5-day schedule. Although we were able to administer mitoxantrone to patients at dose levels of 3.4 and 4.0 mg/m^2 daily $\times$ 5 without grave complications, it became evident that serious leukopenia (grade 3; wbc count nadir < 2000/mm^3) had occurred in more than one half of the instances at the lower dose level of 2.73 mg/m^2 (table 2). We have therefore concluded that the maximum tolerated dose over a 5-day course in this study was 2.73 mg/m^2, with leukopenia being the limiting toxic reaction. Thrombocytopenia was infrequent. The nadir of the myelosuppression occurred at Day 10, with full recovery of the wbc and platelet counts by 2½–3 weeks. Leukopenia appeared to be more evident and pronounced in our patients with extensive liver disease or extensive prior chemotherapy or radiotherapy. Consideration of such factors may indicate a need for dose reduction and caution in administering mitoxantrone in future studies. Minor ECG changes of uncertain relationship to drug therapy were seen after mitoxantrone treatment but were of no clinical importance. Gastrointestinal toxicity was mild and tolerable. Alopecia was minimal.

Mitoxantrone displayed modest antitumor activity in five of 25 patients, most of whom had been heavily pretreated. Although this activity was of interest in this phase I study, it clearly did not meet the usual criteria of clinical significance.

We would recommend further clinical investigation of a 5-day course in phase II studies of mitoxantrone using a dose of 2.73 mg/m^2 in "good-risk" patients, defined as those who do not have extensive liver metastases and have not been heavily pretreated.

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Phase I Clinical Study With 5′-Deoxy-5-fluorouridine, a New Fluoropyrimidine Derivative[1,2]

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SUMMARY

5′-Deoxy-5-fluorouridine (DFUR) is a new fluoropyrimidine derivative with significant antineoplastic activity in animal systems. Compared to 5-FU or other fluoropyrimidines, DFUR has a more favorable therapeutic ratio in Sarcoma 180-bearing mice. DFUR was studied in this phase I trial with a daily × 5 bolus iv injection. A second course was given ≥ 3 weeks after the first day of treatment. Doses were escalated from 300 to 5000 mg/m²/day in 30 patients. Dose-limiting factors were myelosuppression and stomatitis. Hematologic toxic effects were particularly marked on granulocytes. Thrombocytopenia was less frequently encountered. Stomatitis was severe at high doses of DFUR. Eleven patients had nausea or moderate vomiting. Drug-induced myocardial injury may exist, since electrocardiogram changes were recorded in two patients. After rapid iv injection, four patients felt hot in the face and pelvis. Other side effects were minimal. With this daily × 5 schedule of administration, the maximum tolerated dose of DFUR appeared to be 5000 mg/m²/day. The dose recommended for further clinical use is 4000 mg/m²/day × 5 for patients previously untreated with chemotherapy.

[Cancer Treat Rep 66:1307–1313, 1982]

5-FU is an antimetabolite extensively used in the treatment of a variety of tumors since 1957 (1). Its toxic effects consist mainly of gastrointestinal intolerance and mucosal lesions, myelosuppression, alopecia, and dermatitis (2). These effects thus limit the amount of drug that may safely be given to patients. Analogs of this class of agents with a possibly higher therapeutic index or improved level of activity have been sought. 5′-Deoxy-5-fluorouridine (DFUR) is a recently synthesized fluoropyrimidine derivative (3). Its chemical structure consists of a 5-FU molecule attached to a pseudopentose. Various other fluoropyrimidine nucleosides, such as 1-(tetrahydrofuryl)-5-fluorouracil (ftorafur) or 2′-deoxy-5-fluorouridine (FUDR), differ in the chemical structure of the sugar moiety (fig 1).

We have chosen to investigate DFUR in a phase I trial, since this compound has shown marked antineoplastic activity against a wide variety of leukemias and solid tumors in animals. Activity has been detected after ip and oral administration (4,5). As compared to other fluoropyrimidines, DFUR possesses a higher therapeutic index in animals. In Sarcoma 180-bearing mice, 50 mg/kg of 5-FU given ip daily for 10 days induces a leukopenia of 1.05×10^9 cells/liter. This dose level achieves a tumor growth inhibition of 75% as compared to the tumor growth rate in control animals. In the same model, DFUR induces a leukopenia $< 4.0 \times 10^9$ cells/liter only after doses > 400 mg/kg and inhibits tumor growth to a larger extent. Bollag and Hartmann (5) have further

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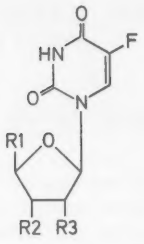

compound	R1	R2	R3
1-(tetrahydrofuryl)-5-fluorouracil	H	H	H
2'-deoxy-5-fluorouridine	CH_2OH	OH	H
5'-deoxy-5-fluorouridine (DFUR)	CH_3	OH	OH

FIGURE 1.—Chemical structure of some fluoropyrimidine nucleosides.

shown that myelosuppressive doses of DFUR are more potent than equitoxic doses of FUDR or ftorafur. Finally, the spectrum of antitumor activity may be different for DFUR than for the other fluoropyrimidines (5).

Animal toxicity studies have been completed in F. Hoffmann-La Roche and Co Laboratories, Basel, Switzerland (6). LD10 for DFUR in normal mice is 543 mg/kg for single iv injection and 360 mg/kg after five daily ip injections. Corresponding doses for rats are > 2000 and 560 mg/kg, respectively. In comparison, the LD10 values for 5-FU are 22 and 24 mg/kg in rats and mice, respectively. In dogs, DFUR and 5-FU induce neurologic symptoms and death after low doses. Gastrointestinal mucosa and endocardium hemorrhages are seen with both compounds.

Transient leukopenia and anemia are observed in rats after prolonged daily administration of DFUR doses > 300 mg/kg. Myelosuppression is also induced by 5-FU, but at daily doses of 10–20 mg/kg. Baboons receiving prolonged daily iv administration do not exhibit leukopenia after DFUR doses up to 80 mg/kg, whereas severe bone marrow toxicity is reported after 5-FU doses of 10 mg/kg. In conclusion, the toxic effects related to DFUR are very similar to those observed after 5-FU administration. However, the doses of DFUR necessary to induce toxic effects are much higher, on a weight basis, than the doses of 5-FU.

In comparison to FUDR, DFUR lacks the hydroxyl group in the 5′ position of its pseudopentose. This group appears necessary for phosphorylation and the direct formation of nucleotides (7). This chemical property implies that either DFUR is directly active or that transformation into 5-FU is necessary for its activity. This transformation has been shown to occur with FUDR (7) and ftorafur (8).

The exact mechanisms of action of DFUR are not yet known. DFUR is a substrate for an intracellular nucleoside phosphorylase. Under the action of this enzyme, 5-FU and other phosphorylated metabolites have been rapidly detected in Ehrlich ascites tumor cells (9). Phosphorylase activity is particularly high in Sarcoma 180 tumor cells as compared to normal mouse cells, with the exception of small intestinal tissue (6). Phosphorylase activity variation among different cell types and/or particular metabolic activation may explain the better tolerance of normal tissue to DFUR as compared to other fluoropyrimidines. This seems of special importance for the bone marrow, explaining the higher myelotoxicity of 5-FU compared to the same dose of DFUR.

MATERIALS AND METHODS

Patients with advanced malignancies were eligible for the trial if they had a leukocyte count > 3.5 × 10^9/liter, a platelet count > 120 × 10^9/liter, a serum bilirubin < 20 μmols/liter, and a serum creatinine < 120 μmols/liter. Performance index had to be > 40 on the Karnofsky scale. Further, expected followup compliance and patient informed consent were required.

DFUR was supplied by F. Hoffmann-La Roche and Co, Ltd, Basel, Switzerland, in vials containing 1000 mg of pure substance as a 10% sodium salt. Immediately prior to use, vials were dissolved with 10 ml of sterile water for injection. DFUR was given daily for 5 days by a bolus iv injection of the solution (100 mg/ml). A second course was administered on Day 22 or later after recovery from toxicity. This

daily × 5 schedule was chosen because it is widely used for 5-FU and thus allows a comparison of clinical tolerance between fluoropyrimidine analogs.

The starting dose of DFUR was 300 mg/m²/day, approximately one third of the mouse LD10 (10). An original scheme was used to rapidly escalate the DFUR doses after the first step (table 1). We anticipated from animal data that the maximum tolerated dose in man should be ten to 15 times higher than the corresponding dose for 5-FU. No intrapatient dose escalation was allowed in this trial. Patients were carefully monitored for signs of clinical toxic effects at least once a week. A complete hemogram was scheduled twice a week during the treatment period, with a blood chemistry weekly. The lowest hematologic counts were separately reported for each patient in the first and subsequent courses of treatment with DFUR. An ECG and audiogram were required before and after completion of the trial. Tumor response was determined after at least two courses with DFUR in patients with measurable or evaluable lesions, using standard criteria of response (11).

The pharmacokinetics of the unchanged drug was also studied. Ten blood samples were drawn into potassium oxalate-containing vacuum tubes at selected times during the first 4 hours after the DFUR bolus injection of Day 1. Samples were also taken after 24 and 96 hours. Test tubes were placed on ice and centrifuged within 1 hour at 4°C. Plasma was then transferred into glass tubes, immediately frozen at –20°C, and stored until drug measurement. At this time, 1 ml of plasma was diluted with an equal volume of saturated ammonium sulfate solution and extracted twice with six volumes of ethyl acetate. The organic phases were then combined and evaporated. The dry extract was later diluted in 1 ml of a mixture of water/methanol/acetic acid (94/6/0.1 vol/vol). An equivalent 80 μl of plasma was subjected to high-performance liquid chromatography separation on reverse phase RP18 with water/methanol/acetic acid (94/6/0.1 vol/vol) as eluant. The drug was quantified at 268 nm using an ultraviolet detector. The sensitivity limit of the method was 50 μg/ml of plasma.

TABLE 1.—Escalation scheme for DFUR

Starting dose	DFUR dose (mg/m²/day)	Relative increase above preceding dose level (%)
1 n	300	
3.3 n	1000	233
6.6 n	2000	100
10.0 n	3000	50
13.3 n	4000	33
16.6 n	5000	25

RESULTS

Thirty patients were treated with DFUR. Patient characteristics are shown in table 2. Most of the patients were previously untreated: only four of them had prior chemotherapy, including two who received 5-FU. One patient had been treated with radiation therapy.

Sixty-six 5-day courses of DFUR were administered. Eight patients had only one course of DFUR. No patient received more than four courses. Except at the highest dose level of 5000 mg/m²/day, four to eight patients were entered at each dose level.

Tables 3–6 show the hematologic toxicity induced by DFUR administration. After the first course of treatment, no leukocyte reduction was observed in the first three dose levels, up to 2000 mg/m²/day. At the next step, two patients showed a moderate leukopenia of 2.1 and 3.1 × 10^9 cells/liter. Four of seven patients treated with 4000 mg/m²/day had a leukopenia < 4.0 × 10^9 cells/liter, with two of them having a count < 1.0 × 10^9 cells/liter. At this dose level, the median time to nadir was 18 days, with a range of 9–22 days. Duration of leukopenia ranged from 2 to 6 days after nadir.

The two patients treated with 5000 mg/m²/day had lowest leukocyte counts of 0.6 and 2.4 × 10^9/liter. Granulocytopenia was particularly severe. One of these patients had a gram-negative sepsis, and both patients were treated with broad-spectrum antibiotics while granulocytopenic. Recovery, as determined by leukocyte counts > 4.0 × 10^9/liter, occurred on Days 19 and 27 at this dose level.

Up to 4000 mg/m²/day, leukocyte toxicity induced in subsequent courses with DFUR was not more

TABLE 2.—Patient characteristics

Characteristic	No. of patients
Patients in study	30
Female/male	15/15
Median age in yrs (range): 63 (38–82)	
Median performance index (range): 70 (50–80)	
Primary tumor	
Lung, non-small cell types	6
GI tract adenocarcinoma	20
Other	4
No prior treatment	25
Radiotherapy alone	1
Chemotherapy alone	4

TABLE 3.—DFUR-induced leukocyte toxicity after 1st course of treatment

Dose (mg/m²/day)	No. of patients evaluable/total No. of patients	Median lowest count (range), × 10^9 cells/liter		Median No. of days to nadir (range)
		Leukocytes	Granulocytes	
300	2/5	9.7 (8.8–10.6)	8.3 (7.5–9.1)	8 (6–9)
1000	7/8	7.3 (4.0–11.6)	4.5 (2.8–9.2)	9 (5–22)
2000	4/4	6.2 (3.9–11.0)	3.5 (2.5–4.4)	15 (5–19)
3000	4/4	4.1 (2.1–6.3)	2.2 (0.7–3.9)	22 (18–22)
4000	6/7	2.3 (0.6–5.3)	0.8 (0.1–2.7)	18 (9–22)
5000	2/2	1.5 (0.6–2.4)	0.02 (0.0–0.03)	17 (17)

TABLE 4.—DFUR-induced leukocyte toxicity after subsequent courses of treatment

Dose (mg/m²/day)	No. of patients evaluable/total No. of patients	Median lowest count (range), × 10^9 cells/liter		Median No. of days to nadir (range)
		Leukocytes	Granulocytes	
300	2/5	11.9 (11.3–12.4)	NA*	4 (4)
1000	4/5	5.4 (3.2–6.7)	3.5 (1.7–5.2)	8 (5–19)
2000	4/4	6.1 (3.8–9.2)	3.1 (2.6–5.3)	12 (7–25)
3000	4/4	5.1 (4.1–6.7)	3.8 (2.1–4.5)	20 (5–22)
4000	2/2	4.5 (4.4–4.5)	1.5 (1.3–1.7)	20 (18–22)

*Not available.

severe than in the first course, and the patients could be re-treated with the same dose of DFUR (table 4). The two patients receiving 5000 mg/m²/day were re-treated with 3000 mg/m²/day.

Thrombocytopenia < 100 × 10^9 cells/liter was seen in several patients after the first or subsequent course with DFUR (tables 5 and 6). Platelet toxicity was encountered at low dose levels after the first or second course with DFUR in patients with a poor performance index or renal insufficiency. Thrombocytopenia was recorded in two of seven patients given 4000 mg/m²/day and in one of two patients treated with 5000 mg/m²/day. Platelet recovery to > 100 × 10^9/liter was rapid in every patient, except one who received 300 mg/m² daily × 5 and died on Day 41 from a gastrointestinal hemorrhage while thrombocytopenic. Autopsy revealed ischemic hemorrhagic necrosis of the jejunum.

Nonhematologic toxic reactions induced by DFUR administration are shown in table 7. Gastrointestinal tolerance was good in most patients. However, 11 patients experienced anorexia, nausea, or discrete vomiting.

Stomatitis was observed in six patients, was severe in four of them, and was seen at various dose levels. After 1000 mg/m²/day, two patients developed stomatitis: mucosal ulcerations were observed in one, an 82-year-old patient treated in error, while having a threefold increase of serum creatinine. The two patients receiving 5000 mg/m²/day developed severe stomatitis with ulcerations. They had no known risk factors.

Precordial pain, appearing during the days of DFUR administration, was observed in two patients treated with 1000 mg/m²/day. ECG changes consisting of ST segment displacements were recorded, and one patient had a significant transient cardiac enzyme elevation. No similar toxic reaction was observed after higher doses of DFUR.

Neurologic symptoms were observed in three patients. Transient tremor, ataxia, and somnolence were seen after 300 and 3000 mg/m²/day of DFUR,

TABLE 5.—DFUR-induced platelet toxicity after 1st course of treatment

Dose (mg/m²/day)	No. of patients evaluable/total No. of patients	Lowest count, × 10⁹ cells/liter		No. of days to nadir	
		Median	Range	Median	Range
300	2/5	389	173–605	8	6–9
1000	7/8	234	62–319	14	5–19
2000	4/4	217	164–380	15	5–21
3000	4/4	185	167–200	15	9–18
4000	5/7	120	30–204	12	9–19
5000	2/2	108	29–186	16	15–17

TABLE 6.—DFUR-induced platelet toxicity after subsequent courses of treatment

Dose (mg/m²/day)	No. of patients evaluable/total No. of patients	Lowest count, × 10⁹ cells/liter		No. of days to nadir	
		Median	Range	Median	Range
300	2/5	133	58–207	12	10–14
1000	4/5	195	88–208	12	5–19
2000	4/4	196	157–312	14	10–21
3000	4/4	162	105–260	6	2–9
4000	2/2	134	131–137	9	8–10

TABLE 7.—DFUR-induced nonhematologic toxic reactions

Type of toxic reaction	No. of toxic reactions at each dose of DFUR (mg/m²/day)					
	300	1000	2000	3000	4000	5000
Nausea and vomiting	1	1	3	2	3	1
Stomatitis	0	2	1	0	1	2
ECG changes	0	2	0	0	0	0
CNS symptoms	2	0	0	1	0	0
Sensation of heat	0	0	1	0	2	1
Alopecia	0	0	0	0	0	1
No. of patients with toxic reactions/ total No. of patients	3/5	4/8	4/4	3/4	5/7	2/2

respectively. Audiogram changes with loss of high frequency detection were observed in one of only four evaluable patients.

Four patients reported a sensation of heat in the face and pelvis. This phenomenon appeared only after very rapid iv injection of at least 2000 mg/m²/day.

Finally, complete alopecia was seen in one patient treated with 5000 mg/m²/day. No vein induration or skin discoloration secondary to treatment was seen.

A total of 12 patients were evaluable for response to DFUR. Antitumor effect was observed in two previously untreated patients who received 2000 mg/m²/day of DFUR. The first patient had < 50% regression of hepatic metastases of a gastric adenocarcinoma, as measured by liver ultrasonography; the second had an almost complete disappearance of a pleural effusion from an adenocarcinoma of the lung, without drainage of the pleural cavity. Tumor stabilization was seen in seven patients after documented disease progression. One of these patients with a colon adenocarcinoma had responded previously to 5-FU.

Seven patients died before completion of two courses of DFUR. Two deaths were related to infectious complications secondary to granulocytopenia in patients treated with 4000 mg/m²/day. As mentioned above, one patient died from massive gastrointestinal bleeding. All of the other deaths were attributable to tumor progression.

In summary, DFUR doses in the range of 4000–5000 mg/m²/day led to severe hematologic and nonhematologic side effects. We felt that further treatment with 5000 mg/m²/day was not warranted. Dose-limiting factors were clearly myelosuppression and stomatitis. We thus considered that the dose of 5000

TABLE 8.—Pharmacokinetic parameters after DFUR iv bolus injection

Parameter	Dose (mg/m²/day)				
	600 (2 patients)	1000 (3 patients)	2000 (3 patients)	3000 (2 patients)	4000 (2 patients)
Mean terminal half-life (mins)	20.5	16.5	25.8	23.4	22.8
Mean distribution volume (liters)	30	18	21.3	26	17
Mean area under the curve (μg/ml × hr)	10.2	30	85.9	112	220
Mean clearance (liters/hr)	127	83	39	42	30

mg/m²/day was the maximum tolerated dose for man when using a daily × 5 schedule for administration of DFUR.

The pharmacokinetics of the unchanged drug has been studied in 12 patients during the first day of DFUR administration. Dose levels have ranged from 600 to 4000 mg/m²/day. Pharmacokinetic data for the unchanged DFUR are the best fit based on a two-compartment open model. The beta half-life values for doses up to 4000 mg/m²/day range from 16.5 to 25.8 minutes (mean, 21.8 ± 3.5) (table 8). This short half-life is independent of the dose of DFUR. On the other hand, the area under the curve increases unproportionally with the dose. As a consequence, the total body clearance does not remain constant but diminishes with escalating doses.

DISCUSSION

Several fluoropyrimidine analogs have been proposed in recent years in the search for a drug with a better therapeutic index and an increased level or broader spectrum of activity in comparison to 5-FU. Overall results have not shown significant advantages of any of these derivatives. Ftorafur does not yield improved activity over 5-FU and has severe toxic effects. Central nervous system side effects, which are dose-limiting, have been correlated with sustained cerebrospinal fluid drug levels (12).

The results of our phase I trial with DFUR show that myelosuppression is induced by this new fluoropyrimidine derivative at doses > 2000 mg/m²/day × 5. This compound appears to be more toxic for leukocytes than for platelets at the dose levels tested. Within a narrow range of dose increments from 3000 to 5000 mg/m²/day, a leukopenia < 4.0×10^9 cells/liter is observed in most patients. Granulocytes are particularly affected. Treatment with DFUR is thus limited by a dose-related leukopenia. Our results do not show evidence for a cumulative myelosuppression after repeated courses with DFUR.

On the other hand, nonhematologic effects seem acceptable. Gastrointestinal tolerance has been good in most patients. However, stomatitis has been seen and is a dose-limiting factor in DFUR treatment. Sensation of heat in the face and pelvis has been observed after rapid iv administration. Similar symptoms have been reported for another 5-FU derivative, 1-hexylcarbamoyl-5-fluorouracil, after oral administration (13). Further cardiac evaluation should be done since DFUR may induce myocardial damage, as evidenced by ECG changes in two of our patients. Cardiac toxicity has also been reported for 5-FU (14,15). Finally, alopecia has been observed in our patients. In summary, DFUR induces nonhematologic side effects closely related to those reported after 5-FU administration. However, the doses necessary to induce these toxic effects are much higher than those of 5-FU.

In conclusion, DFUR can be given at higher doses than 5-FU with an acceptable tolerance. Its toxicity seems to be strongly dose-related, since small dose modifications may sharply increase the severity of the drug-induced toxic reactions. In the search for new active antitumor compounds, DFUR warrants further clinical trial. We propose a dose of 4000 mg/m²/day × 5 every 3–4 weeks for phase II trials in patients untreated previously with chemotherapy. We think that the antineoplastic activity of DFUR has to be established first in tumor types usually sensitive to 5-FU, such as gastrointestinal tumors. The Swiss Group for Clinical Cancer Research (SAKK) has initiated a phase II trial with DFUR in advanced colorectal adenocarcinomas. The activity of DFUR in other tumor types should also be assessed later.

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Activity of Isotretinoin Against Squamous Cell Cancers and Preneoplastic Lesions[1,2]

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SUMMARY

We investigated the effect of the synthetic vitamin A derivative isotretinoin (13-*cis*-retinoic acid) on advanced cancers in 103 patients and on preneoplastic lesions in five patients. Six of 14 patients with squamous cell epithelial cancers had objective regressions of skin or subcutaneous metastases. Three of five patients with preneoplastic lesions had objective responses. The major dose-limiting toxic effects were reversible dermatitis, emotional lability, and headaches. We conclude that the growth of some squamous cell epithelial malignancies can be inhibited by isotretinoin and suggest that other retinoids should be evaluated as antitumor agents.

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Vitamin A is necessary for the normal development of all epithelial tissues (1). This vitamin and its natural and synthetic derivatives (retinoids) have shown significant activity as chemopreventive agents in numerous animal systems, whether the tumor was initiated by chemical or by physical carcinogens (2,3). The retinoids have also shown antiproliferative activity against a large number of animal and human malignant cell lines (4–9). The growth-suppressive effect against cell lines from melanoma (7–9), teratocarcinoma (10), and promyelocytic leukemia (11) has been accompanied by differentiation. In tests on fresh biopsy material from human tumors, we have also observed that retinoids can sometimes inhibit tumor colony formation in soft agar (12,13). Furthermore, several of the retinoids, including the synthetic vitamin A derivative isotretinoin (13-*cis*-retinoic acid), have shown antiproliferative activity against such dermatologic conditions as psoriasis, rare epithelial dysplasias, and basal cell carcinomas (14,15). No serious cytotoxic effects were recorded in these investigations or in the treatment of a large number of patients with conglobate acne (16).

These results suggest that in addition to chemopreventive effects retinoids may have antineoplastic activity. We have therefore conducted a broad phase II trial of isotretinoin to assess its antineoplastic activity and have detected activity in preneoplastic and neoplastic lesions of squamous cell histology.

MATERIALS AND METHODS

Patient characteristics.—Patients were eligible for treatment with isotretinoin if they had advanced cancer or aggressive preneoplastic conditions with measurable disease that could be evaluated for regression. Patients with advanced cancer received isotretinoin at a dose of 3 mg/kg/day orally, while patients with preneoplastic lesions were started at a dose of 2 mg/kg. These doses were empirically selected based on the extensive experience with iso-

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TABLE 1.—Patient distribution and response rate of eligible and evaluable patients treated with isotretinoin

Histologic class	No. of patients	No. (%) of responses*	
		Including mixed responses	Excluding mixed responses
Epithelial†			
Squamous cell			
Advanced	24	6 (25)	2 (8)
Low-grade (preneoplastic)	5	3 (60)	3 (60)
Non-squamous cell	45	1 (2)	1 (2)
Malignant melanoma	13	1 (7)	1 (7)
Nonepithelial†	21	0	0

*Responses include only mixed, partial, and complete; improvements are not included.

†Tumor types included: epithelial squamous cell, high-grade (8 lung, 10 head and neck, and 6 other); epithelial squamous cell, low-grade (3 multiple basal cell, 1 keratoacanthoma, and 1 epidermal dysplasia verruciformis); epithelial non-squamous cell (8 breast, 12 ovarian, 12 colon, 4 bladder, and 9 miscellaneous); and nonepithelial (3 choriocarcinoma, 5 lymphoma, 4 leukemia, and 9 miscellaneous).

tretinoin against dermatologic lesions (14–16). In a pilot study, doses were escalated by 0.5-mg/kg increments every 1–2 weeks to limiting toxicity in the first 16 patients with advanced cancer. It became clear that most patients (12 of 16) would not tolerate > 4 mg/kg of isotretinoin for much longer than 3–4 weeks. Therefore, subsequent patients were started and maintained at a dose of 3 mg/kg of retinoid, and the toxic effects cited represent side effects at this level of drug. To be eligible for evaluation, patients could not have received chemotherapy within 4 weeks or radiation therapy within 16 weeks of entering the study, and they had to receive daily therapy with isotretinoin for a minimum of 1 month.

Response criteria.—Response criteria were the following: *complete response*—the disappearance of all measurable disease; *partial response*—a > 50% decrease of the sum of the products of the perpendicular diameters of all measurable lesions; *mixed response*—a > 50% decrease in a measurable disease site while other sites of disease showed no regression; *improvement (minor response)*—a > 25% but < 50% decrease in total measurable disease; and *progression*—a > 25% increase of any disease parameter.

Evaluation for toxicity.—Patients were serially monitored. A careful physical examination was done every 2 weeks, and liver function studies (LDH, SGOT, SGPT, bilirubin), cbc, and prothrombin time were done monthly. These studies were also repeated when the patient discontinued therapy. A radionuclide liver/spleen scan was obtained in all patients who had been receiving therapy for at least 4 months and was repeated every 4 months thereafter.

RESULTS

A total of 143 patients were entered in this investigation. Thirty-five patients were considered ineligible or inevaluable for the following reasons: no objectively evaluable disease (18 patients), early death (nine), discontinuation of isotretinoin after 2–3 weeks secondary to "rapid progression" (five), and intolerable cutaneous side effects (three).

Results are reported for 108 eligible and evaluable patients. The patient distribution and responses were grouped by histologic class (table 1). Considerable activity of isotretinoin was seen in patients with squamous cell epithelial disease (table 1). The six objective responses in cancer patients and the responses of the three patients with preneoplastic lesions are detailed in table 2. All responses occurred in subcutaneous or skin disease sites. In 45 patients with non-squamous cell epithelial malignancies, one partial response was seen in a patient with ovarian cancer which included a > 50% decrease in a large pelvic mass and complete disappearance of refractory ascites. This response lasted for 2 months, when the patient discontinued therapy unexpectedly on her own volition. Two minor responses (1-month duration) of skin disease from breast cancer were also seen.

Thirteen patients with metastatic malignant melanoma were treated, and one patient had a > 50% decrease in a large (13 × 10-cm) subcutaneous mass

TABLE 2.—Details of responses of patients with neoplastic or preneoplastic squamous cell disorders to isotretinoin

Patient No.	Diagnosis	Prior treatment	Response description	Response	Response duration (mos)
			Neoplastic Disorders		
97	Squamous cell, nasopharynx	5 drugs + radiation therapy	50% decrease in 8 × 5-cm neck mass	Partial	2
123	Squamous cell, epiglottis	Methotrexate	75% decrease in massive (> 12 × 10-cm) neck and jaw mass; resolution of periorbital edema	Partial	2
139	Squamous cell, lung	Radiation therapy	50% decrease in 7 × 9-cm jaw mass; bone lesions stable	Mixed	2
145	Squamous cell, lung	Radiation therapy	Disappearance of axillary lesion (2 × 3-cm); no change in opacified chest roentgenogram	Mixed	7
80	Squamous cell, ear	Radiation therapy	Disappearance of 3 finger lesions and 1 arm lesion; other lesions stable	Mixed	1
3	Squamous cell, face	Multiple surgery, radiation therapy	Remodeling of face; stable orbital lesion	Mixed	10
			Preneoplastic Disorders		
109	Keratoacanthomas	Multiple surgery	Disappearance of 24 of 25 lesions, resected (0.5–2-cm)	Partial	12+
43	Multiple basal carcinomas	Concurrent radiation to 2 large face lesions	Complete response of several unirradiated areas; partial response of additional areas	Partial	16+
154	Epidermal dysplasia verruciformis	None	Resolution of 50% of total mass of multiple lesions	Partial	2*

*Discontinued due to skin toxicity; prompt relapse followed.

for 4 months. No responses were seen in 21 patients with nonepithelial malignancies. The pretreatment characteristics of the patients who responded to isotretinoin did not appear to vary significantly from those of patients who did not respond. Careful stratification and more extensive studies of patients with squamous cell malignancies and malignant melanoma are needed and will be required to substantiate this conclusion.

The side effects of isotretinoin at 3 mg/kg are shown in table 3. No bone marrow or hepatic toxicity was recorded. Nausea and vomiting were very mild. Two patients discontinued therapy secondary to skin toxicity after 6 weeks of therapy, and four patients discontinued therapy secondary to emotional lability or headaches after 6, 8, 12, and 16 weeks of therapy. Twenty-three patients also had serial lipid value measurements. Increases in plasma triglyceride levels were recorded in 12 patients. Isotretinoin was not discontinued, and in six patients who lived long enough, additional serum lipid values were obtained 1 month after completing therapy. Plasma triglyceride levels had returned to normal.

DISCUSSION

In the past decade, therapeutic activity of isotretinoin has been reported in numerous dermatologic conditions (15–17). The present study suggests that isotretinoin has antitumor activity in advanced squamous cell epithelial cancers. Our results also confirm that isotretinoin has antiproliferative activity in preneoplastic lesions such as keratoacanthomas (18) and basal cell carcinoma (15). Bollag (19) had noted previously that epithelial tumors of the skin were affected by retinoids. The responses documented here demonstrate that isotretinoin can also inhibit neoplastic squamous cell epithelial lesions in cutaneous and subcutaneous sites. The site selectivity of many of the responses is of interest and warrants further investigation with regard to mechanism.

The biologic actions of retinoids are probably mediated both indirectly by host cells (20,21) and by a number of mechanisms (22). Data suggest that a major effect is modulated through an intracellular binding protein. This receptor molecule has been

TABLE 3.—Toxicity of isotretinoin in phase II trial

Type of effect	Patient reaction
Bone marrow	None
Hepatic	None
Joint pain	Mild (10%–15%)
Nausea and vomiting	6 patients (mild); 2 patients discontinued
Skin and mucosa*	Mild (95%); 2 patients discontinued, 6 patients lowered dose
CNS	Mild emotional changes or headaches (25%); 4 patients discontinued
Lipid	25% increase in triglyceride level in 12 of 23 patients followed serially

*Cheilitis, dermatitis, conjunctivitis.

identified in many human tumors (23–26); however, in some experimental systems the antiproliferative effect of retinoic acid has not always been associated with the presence of retinoic acid-binding protein (6).

The proportion of ineligible and inevaluable patients was high (24%) compared to our usual rate of 5%–7% in phase II investigations. The large number of patients without objectively evaluable lesions (12.6%) or with early death (6.3%) reflected the attitude of "it's only a vitamin and can't harm the patient," even among a staff that was relatively sophisticated about retinoids. Three percent of the patients appeared to have increased progression of their disease, an incidence that is similar to other phase II studies and probably represents the natural history of the underlying disease process. Nevertheless, isotretinoin may in part function as a hormone, and careful observation for this phenomenon should be made in phase III studies.

The toxicity of isotretinoin was different qualitatively and quantitatively from that of most cytotoxic anticancer drugs. The predominant limiting side effects occurred in skin. Additionally, mild CNS changes consisting of emotional lability and headaches were frequent; this finding was anticipated, as similar changes with retinyl palmitate have previously been reported (27). However, these symptoms are different than those seen in patients with dermatologic conditions, and the presence of headaches and emotional lability probably reflects the higher dose used in our studies. Perhaps of relevance is that retinoids are known to concentrate in the brain (28). In contrast to vitamin A, isotretinoin produced no abnormalities in liver function, a finding consistent with the lack of retention of isotretinoin by the liver (28). The increase in triglycerides, which was seen in the 12 of 23 patients in whom this lipid was measured, is similar to results obtained in patients with dermatologic conditions (29). This effect appears to be dose-dependent, is rapidly reversible upon retinoid discontinuation, and does not involve lipid subclasses correlated with atherosclerotic disease. Nevertheless, cancer patients who will receive retinoids long-term should be carefully monitored for lipid abnormalities.

Many retinoids have been synthesized (4). In dermatologic conditions, isotretinoin and an aromatic derivative (etretinate) have a totally different spectrum of activity (19). We used isotretinoin in this trial because its toxicity had already been studied and found to be acceptable in noncancerous conditions, and an oral formulation was available. Our results in squamous cell neoplasms suggest that other retinoids should also be evaluated for antitumor activity (30,31). Additionally, the evidence of isotretinoin activity that we observed in squamous cell epithelial malignancies provides support for the use of retinoids in additional studies of some of these tumor types as well as in chemoprevention studies.

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Clinical and Clinical Pharmacologic Studies of Aziridinylbenzoquinone[1,2,3]

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SUMMARY

Thirty patients were entered in a phase I trial of aziridinylbenzoquinone (AZQ). These patients were treated with a single iv dose of AZQ daily for 5 days at doses ranging from 0.5 mg/m² to 10.0 mg/m². The dose-limiting and only significant toxic effects were leukopenia and thrombocytopenia, which appeared with relative consistency at iv dosages of 4.0–4.5 mg/m²/day × 5 in patients with extensive prior treatment and 7.0 mg/m²/day × 5 in patients with little prior treatment. One patient with colon cancer achieved a partial response of 4+ months. No other evidence of antitumor activity was observed. Pharmacologic studies revealed that the drug disappeared from the plasma in a biphasic manner (α-half-life = 1.5 minutes, β-half-life = 18 minutes). With our method of analysis by high-performance liquid chromatography, no metabolites were found in the serum.

[Cancer Treat Rep 66:1321–1325, 1982]

Aziridinylbenzoquinone (AZQ) is of interest for a phase I clinical trial for two major reasons. (*a*) AZQ has significant activity against a number of transplantable murine tumors including L1210 lymphoid leukemia, P388 leukemia, Colon 26 tumor, and CD8F mammary tumor (1,2); and (*b*) AZQ possesses a chemical structure in which the properties of lipid solubility, low ionization, and water solubility are optimized (1–4). Both lipid solubility and low ionization are believed to be important in the penetration of drugs into the central nervous system (5). Not only does AZQ penetrate and attain significant concentration in the central nervous system of animals (6), but it also has shown significant antitumor activity against intracranially implanted L1210 and P388 leukemias and an ependymoblastoma when given ip (1,2).

At the present time the exact mechanism of action of AZQ is unknown, although it is thought to possess alkylating activity against DNA (1,7). An additional mechanism of activity of this drug may involve free-radical formation, which could account for some of its antitumor activity (8). Preclinical toxicologic studies in mice, dogs, and monkeys (1) indicated that the major toxicity of AZQ was in the gastrointestinal, hematopoietic, and lymphatic systems; lesser toxicity was noted in the respiratory, hepatic, nervous, and renal systems. The schedule of treatments appeared to have little effect on the toxicity. With a 5-day schedule, the toxic dose low for the most sensitive species studied (beagle dog) was 1.6 mg/m². The present phase I study was undertaken to determine the characteristics of toxicity, the minimum dose that produces consistent reproducible evidence of biologic effect, and the maximally tolerated dose of AZQ in humans, when given iv daily for a 5-day course.

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[5]We are grateful for the skillful assistance of Cynthia Ackerley in HPLC analysis and Martha Dewees in clinical data management.

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MATERIALS AND METHODS

All patients selected for this study had advanced, histologically proven cancer for which conventional therapy had been exhausted or for which there was no conventional or investigational therapy with a reasonable expectation of benefit. AZQ was administered in the wards and clinics of the Medical Center Hospital of Vermont. The nature of the study was explained to each patient, who signed an appropriate informed consent before the study began. Patients who entered the study had a life expectancy of at least 8 weeks, had a Karnofsky performance status ≥ 30, and were > 18 years of age.

AZQ was given iv. Each 10-mg vial was reconstituted with 0.5 ml of sterile *N*,*N*-dimethylacetamide and 9.5 ml of sterile 0.01 M phosphate buffer (pH 6.5), to a final concentration of 1 mg/ml. The daily dose was further diluted to 20 ml with normal saline and given by iv push over a period of 30–60 seconds. The daily dose was repeated for 5 days, and each course was repeated no more frequently than every 3 weeks. All patients were treated until toxic effects were observed. If there was evidence of therapeutic response, the drug was continued until disease progression was demonstrated. The starting dose was 0.5 mg/m^2/day $\times$ 5 (⅓ the toxic dose low in the dog) and dose escalation followed a modified Fibonacci scheme. Baseline data included a history, physical examination, and review of the microscopic slides used to make the diagnosis. Initial laboratory studies included a cbc and platelet count, bone marrow examination, urinalysis, biochemistry screening profile,[6] and other studies determined to be necessary on the basis of tumor type and the clinical condition of the patient. Followup studies included discussion of symptoms, physical examination, and cbc, daily while receiving AZQ and twice weekly thereafter. Biochemistry screening and urinalysis were done weekly during and for 3 weeks following AZQ administration. X-rays, scans, etc, were done as necessary to evaluate changes in organ function that might be due to growth or regression of tumor or due to drug toxicity.

The concentration of AZQ in the serum and cerebrospinal fluid was determined by a high-performance liquid chromatography (HPLC) method developed in our laboratories, as has been previously described (9) and is summarized here.

A Spectra Physics model 8000 microprocessor-controlled high-performance liquid chromatograph equipped with a data system was used. The chromatograph was equipped with a Schoeffel model 770 variable-wavelength ultraviolet detector set at 340 nm. The column was an Altex (Berkeley, CA) Ultrasphere IP 5-μm column (25 cm $\times$ 4.6 mm ID). A guard column (7 cm $\times$ 0.2 cm ID) packed with Co:Pell ODS (25–37 μm; Whatman, Clifton, NJ) was installed to protect the 5-μm column. Samples were injected onto the column through a 10-μl loop, using a manual injector. For each time point studied, 10 ml of whole blood was obtained from patients by venipuncture in the arm opposite that used for injection of AZQ. Serum was obtained by centrifugation and stored at –10°C for < 48 hours prior to analysis.

Serum samples were prepared for injection onto the column using Sep-Pak cartridges (Waters Assoc, Milford, MA). The use of the Sep-Paks affords both sample purification and drug concentration. Cartridges were prepared for use as described in the brochure supplied by Waters Assoc. A measured volume (0.5–4.0 ml) of serum or stock drug solution was loaded onto the Sep-Pak, which was then washed with 1.0 ml of HPLC-grade water containing PIC reagent A. AZQ was then eluted by washing the Sep-Pak with 3.0 ml of HPLC-grade methanol containing 5 mM tetrabutylammonium phosphate (PIC reagent A: Waters Assoc). The methanol was removed under nitrogen, and the residue was taken up in a minimum volume (100–200 μl) of HPLC-grade water. The sample was vortexed and then centrifuged to remove the insoluble material. The resulting solution was used directly for injection onto the column.

AZQ was eluted from the column using an isocratic mobile phase of water:methanol (65:35, vol/vol) containing 0.005 M tetrabutylammonium phosphate (PIC reagent A). A flow rate of 1.0 ml/minute was used. The concentration of AZQ in the eluate was measured by ultraviolet detection at 340 nm. Quantitative analysis was based on peak areas and was computed using preset integration programs in the data system of the Spectra Physics HPLC instrument. Prior to sample analysis, the chromatograph was calibrated daily using AZQ solutions of known concentration.

The minimum detectable level of AZQ was 7.0 ng injected, at a detector setting of 0.01 aufs and a recorder attenuation of 0. The signal-to-noise ratio was ≤ 6 under these conditions.

To determine the recovery of AZQ from plasma, samples of human plasma were spiked with known concentrations of AZQ and then extracted using the Sep-Pak cartridges. Even when the plasma samples were spiked with relatively low concentrations of AZQ (eg, 1–3 μg/ml), the recovery of the drug from

[6]Serum chlorides, CO_2, potassium, sodium, urea nitrogen, creatinine, uric acid, glucose, alkaline phosphatase, total and direct bilirubin, SGPT, SGOT, lactic dehydrogenase, total protein, albumin, calcium, phosphorus, and cholesterol.

the plasma was ⩾ 90% as calculated by the data system of the chromatograph. Recovery of AZQ from human serum samples was identical to that obtained in human plasma samples. Blood samples were obtained 1, 2, 5, 10, 20, 30, and 60 minutes after injection of AZQ. Serum AZQ half-times were calculated directly from the data, using least squares regression linear analysis.

RESULTS

Clinical Studies

Thirty patients (table 1) were enrolled in the study (23 evaluable). A total of 46 courses of AZQ were given (37 were evaluable; eight courses were inevaluable due to early death from disease before toxicity could have occurred). The major dose-limiting toxic effect was bone marrow depression (table 2). In patients with extensive prior chemotherapy and/or radiotherapy (> 6 months of prior chemotherapy, > 2 months of chemotherapy and/or large-field radiotherapy, or five or more radiation therapy fields), this toxic effect occurred relatively consistently at a dose of 4.0–4.5 mg/m²/day × 5, while in patients with little prior treatment, it did not occur with relative consistency until a dose of 7.0 mg/m²/day × 5 was reached. All patients treated at a dose of 10 mg/m²/day × 5 developed severe leukopenia or thrombocytopenia; the median wbc count nadir occurred on Day 21, and the median platelet count nadir occurred on Day 20. In this study, there were no drug-related renal, hepatic, respiratory, mucosal, central nervous system, or cardiac toxic effects, and there was no alopecia or nausea and vomiting. Sixteen of the 46 courses were preceded by one to three prior courses at the same or lower doses. These revealed no definite evidence of cumulative toxicity. Since no patients had abnormal renal function and only one patient had a transient increase in bilirubin, we were unable to draw any conclusions about the effect of these variables on AZQ toxicity. The tumor types treated in this study are listed in table 1. One patient with colon cancer achieved a partial response for 4+ months (a > 50% decrease in the product of two perpendicular diameters of a palpable rectal mass and a > 30% decrease in the total size of the liver in the midclavicular line). None of the other patients showed evidence of response.

TABLE 1.—Characteristics of patients given AZQ

Characteristic	No. of patients
Age in yrs	
Median: 59.5	
Range: 23–78	
Sex	
Males	19
Females	11
Prior chemotherapy	17
Performance status	
Median: 70	
Range: 40–100	
Tumor types	
Lung	11
Adenocarcinoma	6
Large cell carcinoma	3
Small cell carcinoma	1
Squamous cell carcinoma	1
Colorectal carcinoma	5
Breast carcinoma	3
Renal cell carcinoma	2
Malignant melanoma	1
Synovial sarcoma	1
Lymphocytic lymphoma	1
Leiomyosarcoma	1
Astrocytoma	1
Adenocarcinoma, endometrium	1
Nodular sclerosing Hodgkin's disease	1
Clear cell carcinoma, vaginal wall	1
Adenocarcinoma, unknown primary	1

Clinical Pharmacologic Studies

The results of these studies are summarized in figures 1 and 2. When given by rapid iv injection (fig 1), AZQ showed a rapid phase of drug distribution (α-half-life = 1.5 minutes) followed by a slower phase of drug elimination (β-half-life = 18 minutes). AZQ could not be detected in blood samples obtained at 24 hours after drug administration. The apparent volume of distribution for AZQ was calculated at 2.1 liters/kg. This large apparent volume of distribution is consistent with that usually associated with lipid-soluble drugs and implies wide distribution, extensive tissue uptake, or both. When AZQ was given by slow iv infusion over a period of 5 minutes (fig 2), the β-half-life was similar to the β-phase estimation for more rapid drug administration (fig 1). This indicates that the elimination phase is not dependent on the rate of drug administration. No metabolites were detected over the 60-minute period examined in this study. It is possible, however, that our method, designed to measure AZQ specifically, fails to detect metabolites that differ substantially from the parent drug with respect to solubility characteristics and chromatographic and spectroscopic properties (9). In one patient given a single iv dose of 15 mg/m², no AZQ was detected in the ventricular CSF obtained 60 minutes after injection of AZQ. Simultaneous serum samples revealed higher drug concentrations but α and β phases similar to those shown in figure 1.

TABLE 2.—Hematopoietic effects of AZQ in patients with little or extensive prior therapy

Dose level ($mg/m^2/day \times 5$)	No. of courses	Median wbc count nadir, cells $\times 10^3/mm^3$ (range)	Median platelet count nadir, cells $\times 10^3/mm^3$ (range)
		Little prior therapy	
0.5	1	—	—
3.0	1	—	—
4.0	2	—	—
4.5	1	—	—
7.0	8	3.3* (0.9–8.5)	40* (16–240)
10.0	3	0.6 (0.5–0.7)	5 (2–16)
		Extensive prior therapy	
0.5	1	—	—
1.0	2	6.7 (4.9–8.5)	114 (58–171)
2.0	2	—	—
3.0	2	4.2 (3.5–5.0)	65 (18–112)
4.0	6	3.5 (2.2–3.7)	76 (26–330)
4.5	2	6.2 (3.6–8.6)	110 (109–112)
7.0	5	1.5† (0.9–2.0)	16† (2–68)
10.0	1	0.4	6

*Based on only 5 patients; 3 others had no changes.
†Based on only 4 patients; 1 other had no change.

DISCUSSION

AZQ was chosen for phase I trial because of its solubility characteristics and because of its activity in a number of murine tumor systems. In this phase I trial of AZQ, the toxicity (myelosuppression) was manageable and, in contrast to preclinical studies, no appreciable gastrointestinal toxicity was found. In addition, we found that the drug rapidly disappears from the serum in a biphasic manner, and in one patient the drug did not appear to penetrate the central nervous system. In view of the preclinical data and because other investigators have been able to detect AZQ in the CSF of nonhuman primates (6) and of patients (10,11) given the same or higher doses of this drug, tumors of the central nervous system should be included in phase II trial. Although we did not demonstrate the presence of AZQ in the single CSF specimen examined, that experience is insufficient, and additional studies of penetration of AZQ into CSF must be done. Since toxicity was restricted to myelosuppression, examination of AZQ in leukemia is warranted, and the limited activity seen in colon cancer in this phase I study suggests further trial in that tumor. On the basis of these toxicity data and the evidence of antitumor activity in one patient with colon cancer, we recommend that a phase II trial be initiated in solid tumors at an iv dose of 7.0 $mg/m^2/day \times 5$ in patients with little prior chemotherapy and/or radiotherapy and at 4.0 $mg/m^2/day \times 5$ in patients with extensive prior treatment. For patients with leukemia we recommend that further studies begin at an iv dose of 10 $mg/m^2/day \times 5$.

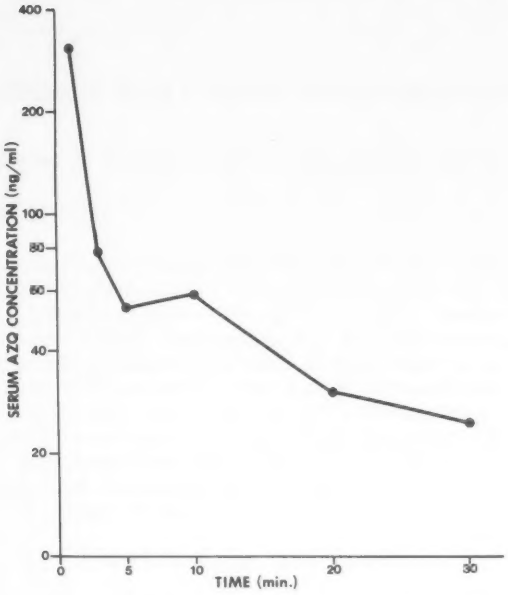

FIGURE 1.—Serum drug concentrations of a single patient given 7.0 mg/m² of AZQ by a rapid (0.5 min) iv injection. Data are presented as means of 3 determinations/time point.

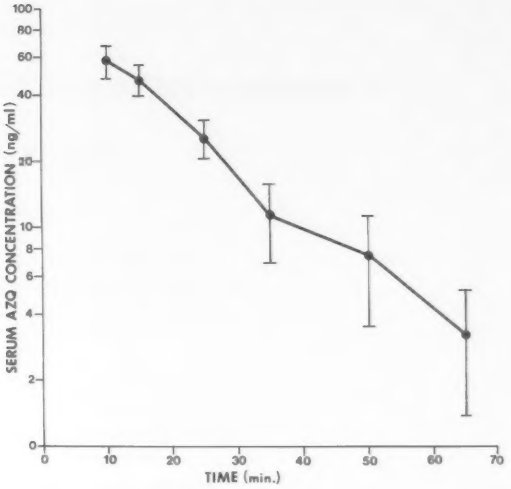

FIGURE 2.—Serum drug concentrations of 11 patients given 7.0 mg/m² of AZQ by a 5-min iv infusion. 9 patients received at least 2 courses of AZQ at this dose level. Data are presented as means ± SE of 11–20 determinations/time point.

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Clinical and Clinical Pharmacologic Studies of Mitoxantrone[1,2]

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SUMMARY

The drug mitoxantrone was given to 31 patients in a phase I clinical trial. The schedule of administration was once weekly for 3 weeks followed by 3 weeks of observation before further treatment. Dose range was from 1.5 to 6 mg/m². Leukopenia was the dose-limiting toxic effect and thrombocytopenia was mild. Partial alopecia occurred in four of 12 patients evaluable for hair loss. Pharmacokinetic studies employing a fluorometric assay based on DNA binding measured by displacement of ethidium bromide revealed a first-phase half-life of about 15 minutes, with a prolonged subsequent phase. A dose of 6 mg/m²/week × 3 was well-tolerated in patients not heavily pretreated with myelosuppressive therapy. Only minor clinical activity was seen with mitoxantrone.

[Cancer Treat Rep 66:1327–1331, 1982]

Mitoxantrone (DHAD) is a new antineoplastic drug developed in an attempt to improve on the clinical effectiveness of currently used anthracyclines (1). The irreversible myocardiopathy which can result from large cumulative doses of doxorubicin limits its use in patients with responsive tumors. In preclinical studies, DHAD has been shown to produce less cardiotoxicity but comparable antitumor effect (2).

Antineoplastic screening studies with DHAD showed activity in P388 and L1210 leukemia, B16 melanoma, and Colon 26 murine tumor systems. Studies in rats, beagle dogs, and cynomolgus monkeys determined that the major toxic effects of DHAD were diarrhea, emesis, and myelosuppression, all findings associated with many other clinically used anticancer agents. No evidence of cardiotoxicity was found clinically or on microscopic examination of myocardium in animals treated with DHAD (2).

MATERIALS AND METHODS

A phase I clinical study was undertaken to determine the toxicity of DHAD given as a single dose weekly for three doses. Pharmacologic studies were also done on some of the treated patients to determine the pharmacokinetics of DHAD and DHAD equivalents.

The patients treated in this study had histologically proven cancer that was not responsive to standard therapy or for which there was no conventional treatment with a reasonable expectation of benefit. Patients were treated in the ambulatory clinic at the Vermont Regional Cancer Center or in the wards of the Medical Center Hospital of Vermont. The histologic types of tumor are listed in table 1. Patient characteristics are shown in table 2.

Pretreatment evaluation included cbc with differential and platelet count, SMA-6 and -12 chemistry profiles, ECG, bone marrow aspirate and biopsy, urinalysis, and physical examination in all patients. Other procedures including plain X-rays necessary for clinical evaluation were done when indicated. Patients with a history of doxorubicin treatment or those who were considered at high risk

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for development of congestive heart failure underwent radionuclide ventriculography to determine the status of myocardial performance. During evaluation each patient had cbc with differential and platelet count three times a week and urinalysis and chemistry profiles weekly.

Each patient was interviewed and examined prior to each dose of DHAD. Informed consent consistent with federal and University of Vermont guidelines was obtained from each patient before entry in the study. No patients were treated with other chemotherapy within 30 days of beginning treatment with DHAD.

DHAD was supplied by the Investigational Drug Branch of the National Cancer Institute (Bethesda, MD) as a sterile solution at a concentration equivalent to 0.5 mg/ml of the free base. The drug was diluted in 50 ml of 5% dextrose in water or normal saline and was injected through the side port of a freely flowing iv line over 5–20 minutes.

The schedule of drug administration was a single injection weekly for 3 weeks followed by 3 weeks of observation prior to the start of a new course. One third of the toxic dose low in the beagle dog, the most sensitive species, was chosen as the initial dose; the dose was 1.5 mg/m^2 for the first patients treated.

Dose escalations followed a modified Fibonacci scheme as outlined in table 3. At least three patients were treated at each dose level. Patients who demonstrated no prohibitive hematologic toxicity and no tumor progression during one course were retreated at the same or higher dose level. When a course of therapy consisted of less than three doses of DHAD a new course was not begun before 3 weeks following the last dose. Thirty-one patients were treated with 51 courses of DHAD, each course of treatment being one, two, or three doses of drug depending on toxicity.

TABLE 1.—Tumor types

Tumor	No. of patients
Non-small cell lung carcinoma	9
Breast carcinoma	7
Colon carcinoma	4
Carcinoma, unknown primary	3
Renal cell carcinoma	2
Ovarian carcinoma	1
Bladder carcinoma	1
Leiomyosarcoma	1
Rhabdomyosarcoma	1
Small cell lung carcinoma	1
Medullary thyroid carcinoma	1
	31

TABLE 2.—Patient characteristics

Characteristic	No. of patients
Total	31
Male	14
Female	17
Median age in yrs (range)	
Male: 51.4 (29–67)	
Female: 59.4 (47–78)	
Prior treatment	
None	6
Chemotherapy	20
Doxorubicin to 400 mg/m^2	7
Radiotherapy	19

Assay of DHAD and Equivalents

The fluorometric assay is carried out as follows: ethidium bromide (Sigma Chemical Co, St. Louis, MO; 50 µl of a 1×10^{-5} M solution in water) is added to pH 7.0 phosphate buffer (2.0 ml) and to this solution is added a sample of serum (usually, 100–200

TABLE 3.—Myelosuppression by DHAD

Dose (mg/m^2)	No. of patients/ No. of courses	Median wbc count (range)*		Median platelet count (range)*	
		Before therapy	After therapy	Before therapy	After therapy
1.5 × 3	3/3	6.5	3.6 (2.7–5.6)	250	124 (117–162)
2.0 × 2	1/1	4.3	3.4	183	108
2.0 × 3	4/4	6.4	5.2 (3.3–11.9)	427	301 (53–595)
4.0 × 1	4/5	5.7	1.9 (1.6–2.8)	235	206 (60–224)
4.0 × 2	5/7	8.5	1.8 (1.0–2.6)	187	120 (70–195)
4.0 × 3	6/8	6.8	2.5 (1.6–5.3)	352	301 (99–455)
6.0 × 2	13/14	6.8	2.1 (1.2–6.4)	330	196 (47†–393)
6.0 × 3	7/11	10.2	3.8 (1.5–87.9)	311	200 (93–460)

*Cells × 10^3/mm^3.
†Patients had thrombocytopenia prior to therapy.

μl); the resulting solution is used as a blank to zero the spectrophotofluorometer (Aminco-Bowman instrument; excitation, 476 nm; emission, 596 nm). DNA (Sigma, type I; 50 μl of a solution containing 2 mg of DNA in 50 ml of pH 7.0 phosphate buffer) is then added and the increment in fluorescence intensity at the indicated wavelength is measured. For serum samples containing DHAD, the expected increase in fluorescence on adding DNA is suppressed in a concentration-dependent manner. A standard curve was generated for each patient studied by adding increments of DHAD (5–50 μl of a 5-μg/ml stock solution in water) to a cuvette containing serum obtained prior to drug administration, buffer, ethidium bromide, and DNA and by recording the decrease in fluorescence that occurred with each increment of DHAD.

RESULTS

Toxicity

Myelosuppression was the major side effect noted, with leukopenia being dose-limiting. The median pretreatment platelet and wbc counts are shown in table 3. Most patients receiving a three-dose course of DHAD experienced a wbc count nadir at approximately Day 20 (median). For those patients treated with a course of 4 or 6 mg/m^2 × 3, the median granulocyte count nadir was 1.4 × 10^3 (range, 0.6–4.2). This nadir occurred at a median of 21 days (range, 15–26).

DHAD appears to be relatively platelet-sparing. In no patient was thrombocytopenia dose-limiting. The median nadir platelet count for each level and the number of doses was always > 50% of the median pretreatment level. In patients treated at the higher doses of DHAD, the degree of wbc suppression was dose-related.

Alopecia occurred in four of 12 patients who were evaluable for that effect. Nineteen patients were not evaluable because of prior chemotherapy or radiotherapy to the scalp which caused alopecia. Of the four patients who had hair loss during DHAD therapy, none had total alopecia. One patient who had no hair loss during DHAD treatment (4.0 mg/m^2 × 3 and 6.0 mg/m^2 × 2) did experience partial alopecia with subsequent doxorubicin administration at 60 mg × 1. Another patient who developed alopecia during a course of cyclophosphamide, cisplatin, and doxorubicin noted regrowth of hair during DHAD therapy (6 mg/m^2 × 2).

Nausea was very mild or absent in all patients. No patient had reproducible vomiting that could be related to DHAD administration. One patient did experience a brief episode of vomiting 20 hours after her second dose of 4.0 mg/m^2. Five patients had mild nausea for about 24 hours after DHAD injection. Two patients complained of feeling cold and tired for 24–48 hours after each dose of DHAD.

At the doses given, no patient had blue discoloration of the sclerae or skin. Urine voided soon after drug administration was frequently noted to have a green color.

No patient had obvious cardiotoxicity secondary to DHAD. The largest cumulative doses in this study were 45 and 36 mg/m^2. One patient who had received 470 mg/m^2 of doxorubicin developed biventricular cardiac failure approximately 2 years after cessation of doxorubicin therapy. The signs and symptoms of congestive heart failure were well-controlled with digoxin and diuretics prior to therapy with DHAD. Treatment with DHAD to a total of 45 mg/m^2 was not associated with clinical worsening of his heart failure.

Response

Two patients exhibited brief clinical responses to DHAD. One woman with metastatic breast cancer previously treated with multiple courses of cytotoxic and hormonal therapy had an objective decrease in the size of numerous small skin metastases. For individually measured lesions the area of tumor reduction was < 50% and within 7 weeks she had bone disease manifested by pain and radiographic changes.

Another patient with metastatic breast cancer had a marked decrease in pain after 2 days of increased pain coincident with DHAD therapy. Her disease was metastatic to the bones and this symptomatic response was identical to that seen with her first treatment with cyclophosphamide, methotrexate, and 5-FU which led to a 1-year partial remission. Despite the initial symptomatic improvement with DHAD, however, she had definite disease progression within 6 weeks and later failed to respond to doxorubicin.

No patients with lung cancer, including seven who had received no prior chemotherapy, showed a response to DHAD.

Pharmacologic Studies

At the time these studies with DHAD were initiated there were no published methods available for analytic studies of this drug in biologic samples. The development and application of high-performance liquid chromatography (HPLC) procedures for DHAD analysis were unsatisfactory and a different approach to analysis was therefore sought. The approach is based on the ability of DHAD to form

intercalative complexes with DNA and involves measurement of the decrease in fluorescence of DNA-ethidium bromide complexes that occurs when DHAD displaces ethidium bromide from the complex. As shown in table 4, when samples of human serum containing DHAD are analyzed by this method a concentration-dependent suppression of fluorescence is observed. The method is capable of detecting the presence of DHAD in serum (and urine), even at the lowest drug dose studied (1.5 mg/m^2). Most of these studies of DHAD levels in serum were carried out on samples obtained when the drug was administered at 6 mg/m^2. Table 5 summarizes these studies; DHAD levels decrease rapidly (estimated half-life = 15 minutes) during the first 30 minutes after completion of drug infusion with a subsequent much slower phase. There is a consistent increase in DHAD (and/or equivalents) when serum levels are analyzed 24 hours after drug administration. This observation may reflect redistribution of DHAD from tissue "stores" or the formation of a DHAD metabolite that is more potent than the parent compound as an intercalator and therefore is detected by this assay as more than one DHAD equivalent. Further studies of the later stage(s) of DHAD elimination from serum clearly are indicated and we plan to carry out such studies, both in experimental animals and in patients participating in phase II studies with DHAD. It is important to note that the "peak" drug levels observed in this study were comparable, taking into account different techniques of administration (ie, infusion versus bolus injection) to those reported recently for a somewhat higher dose of DHAD in man, using ^{14}C-labeled drug and HPLC analysis (3). In addition, studies with the labeled drug also revealed the presence of significant levels of DHAD in the blood at 24 and 72 hours after iv administration of this drug. The estimated initial half-life (rapid phase) for DHAD as well as the suggestion of a prolonged elimination phase that follows the rapid elimination phase are in agreement with observations on the clinical pharmacology of this drug reported by Savaraj et al (4); similar elimination profiles are observed with doxorubicin in patients and in experimental animals (5,6).

TABLE 4.—Inhibition of fluorescence by DHAD in human serum samples*

Concentration of DHAD (μM)	% inhibition of fluorescence
0.15	86.3 ± 6.2
0.10	71.1 ± 4.0
0.075	54.6 ± 3.6
0.050	43.3 ± 3.6
0.025	22.8 ± 2.8

*N = 8. Serum samples were obtained from patients before administration of DHAD. The indicated amounts of DHAD were added to assay solutions containing 100–200 μl of serum.

DISCUSSION

This trial demonstrates that DHAD can be given to patients who have not been heavily pretreated with other chemotherapy at a dose of 6 mg/m^2 weekly for three doses. Reversible myelosuppression is the major toxic effect and there appears to be a relative platelet-sparing effect. Nonmyelosuppressive toxicity was mild at this dose; however, partial alopecia did occur in some patients.

There is laboratory evidence that the mechanism of the antineoplastic action of DHAD is at least in part similar to that of doxorubicin (7). Both drugs bind to DNA and inhibit nucleic acid synthesis. DHAD, however, may have antineoplastic activity on the basis of effects other than DNA intercalation.

If the lack of the amino sugar moiety eliminates or lessens cardiotoxicity for DHAD compared to doxorubicin, then clinical utility will be greatly enhanced. We are unable to comment on the presence or lack of DHAD-induced heart damage but it must be noted that the highest cumulative doses attained in this study do not approach those which would be expected to cause cardiac toxicity. Long-term use will approach such levels and continued evaluation will be necessary.

Patients with a history of prior doxorubicin therapy may develop cardiotoxicity during subsequent DHAD therapy. Several cases of suspected DHAD-induced heart disease have occurred in ongoing phase II trials. Because of the difficulty in determining the etiology of cardiac toxicity in patients who have received both doxorubicin and DHAD, investigators should follow the recommendations of the Investigational Drug Branch of the National Cancer

TABLE 5.—Serum concentration of DHAD (and/or equivalents)

Time*	Concentration (μM)†
0	0
5 mins	0.47 ± 0.06 (0.75)
15 mins	0.31 ± 0.03 (0.50)
30 mins	0.19 ± 0.02 (0.21)
60 mins	0.16 ± 0.03 (0.12)
120 mins	0.15 ± 0.03
24 hrs	0.22 ± 0.04 (0.21)

*"0" is immediately before infusion began; other times are following completion of infusion.

†Includes DHAD and any metabolites that are capable of intercalating into DNA; mean ± SEM. Nos. in parentheses are for a patient given DHAD at 12 mg/m^2 for pharmacologic study; all other values are for 11 patients given 6 mg/m^2.

Institute and restrict eligibility for DHAD therapy to those patients without a history of heart disease and who have not received doxorubicin at a cumulative dose > 400 mg/m² for adults and 450 mg/m² for children.

In a phase I study in which DHAD was given as a single dose every 3 weeks, Von Hoff et al (8) found 12 mg/m² to be the maximum tolerated dose. They, too, failed to see cardiac toxicity, but in their study also, cumulative doses did not reach levels which will be seen with prolonged therapy and which might be expected to cause myocardiopathy. Other phase I trials of DHAD using schedules of weekly (9), daily × 3 every 3 weeks (10), and daily × 5 every 3 weeks (11) drug administration have been reported. In these trials myelosuppression was also the dose-limiting toxic effect.

On the basis of promising preclinical data and good patient tolerance of the drug we would recommend further studies of DHAD, particularly in patients who have not been treated with doxorubicin, to determine cardiotoxicity and antitumor effectiveness. A weekly dose of 6 mg/m² for 3 weeks is a suitable schedule for patients without extensive prior chemotherapy or radiotherapy.

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Plasma and Cerebrospinal Fluid Pharmacokinetics of Acivicin in Ommaya Reservoir-Bearing Rhesus Monkeys[1]

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SUMMARY

Acivicin was administered iv to rhesus monkeys bearing Ommaya reservoirs, and serial blood and cerebrospinal fluid (CSF) samples were collected and analyzed to determine the time course of drug concentrations in these body fluids. After iv doses of 4 or 20 mg/kg (50 or 250 mg/m^2), acivicin plasma concentrations demonstrated a rapid initial decline (distribution phase), and then declined exponentially with a terminal (elimination phase) half-life of 3–4 hrs. CSF concentrations increased over a period of 2–2.5 hrs, reaching peak values of 2.0–2.7 μg/ml at 20 mg/kg and 0.3–0.5 μg/ml at 4 mg/kg; thereafter, CSF levels declined in parallel with plasma, with a CSF/plasma concentration ratio of 0.10–0.17. A three-compartment pharmacokinetic model gave a close fit of predicted and observed plasma and CSF concentration data. Significant and predictable CSF penetration by iv administered acivicin in monkeys is consistent with observation of CNS side effects in patients in the phase I clinical trial and suggests that acivicin should be evaluated in the treatment of CNS malignancies and metastases.

[Cancer Treat Rep 66:1333–1341, 1982]

Acivicin is an amino acid antitumor agent that is currently in phase I clinical trials (1–3). Recently, a report appeared describing CNS toxicity (dysphoria, lethargy, somnolence, ataxia, and hallucinations) as being dose-limiting in patients receiving a single dose every 3 weeks (4). CNS toxicity that was not dose-limiting was also observed in a daily dosing schedule (5). The only CNS effects observed in the dog and monkey toxicity testing done prior to the clinical trial were sedation and disorientation occurring at lethal and superlethal doses that caused death from gastrointestinal toxicity (6).

Acivicin was previously shown to cause significant prolongation of life in mice bearing intracranially inoculated L1210 leukemia (7) and ependymoblastoma (8). During extensive investigations of acivicin disposition and pharmacokinetics, the agent was measured in significant quantities in brain tissue of mice and dogs after parenteral administration (9; J. P. McGovren, unpublished data). Significant distribution of acivicin into brain tissue was unexpected because of the water-soluble, zwitterionic nature of the molecule at physiologic pH.

These findings prompted a kinetic study of the penetration of acivicin into the cerebrospinal fluid (CSF) in Ommaya reservoir-bearing rhesus monkeys. The Ommaya reservoir is a subcutaneously implanted device allowing direct access to the fourth ventricle of the brain for serial CSF sampling or drug administration in unanesthetized animals without disruption of the blood-brain barrier (10–12). The goal of the study was to quantitate the

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time course of acivicin concentrations in the plasma and CSF of an animal model thought to be predictive for the pharmacokinetic behavior of chemotherapeutic drugs in man (12). The findings should be helpful in understanding the CNS effects observed in humans receiving acivicin and in the clinical evaluation of acivicin activity against CNS malignancies and metastases. Such tumors are notably refractory to chemotherapeutic attack due (at least in part) to restricted passage of drugs across the blood-brain barrier (13).

MATERIALS AND METHODS

Acivicin was produced by microbial fermentation and purified at The Upjohn Company (14–16).

Adult male rhesus monkeys (from the NIH Primate Center) weighing 5.4–8.6 kg and bearing previously implanted Ommaya reservoirs were used in the studies. Details of animal preparation and care were as previously described (10–12). Integrity of the reservoir system was monitored by biweekly cell counts and cultures on 3-ml CSF samples.

Pharmacokinetic Studies

Monkeys received doses of 20 or 4 mg/kg (250 or 50 mg/m^2). Two monkeys were studied at each dose. Acivicin was administered as a bolus into the saphenous vein as the appropriate volume of 10 mg/ml of drug in 0.9% NaCl solution. Blood and CSF samples were drawn before treatment and at 15, 30, and 45 mins and 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 9, 12, 24, 28, and 32 hrs after dosing. CSF was withdrawn from the reservoir as previously described (11). Blood samples were taken from the cephalic vein and centrifuged; plasma was then harvested and frozen until assay. To monitor possible drug-related toxicity, blood samples were also taken before treatment and at weekly intervals for the following determinations: cbc including platelet count, prothrombin time, partial thromboplastin time, serum glutamic-pyruvic transaminase, sodium, potassium, chloride, and SMA/12 serum chemistry profile.

Analytic Methods

Acivicin concentrations in monkey plasma and CSF were assayed by a microbiologic method using *Bacillus subtilis* UC-902 cultivated in a completely synthetic medium (16). Samples were assayed full strength or diluted in 0.1 M Na_2HPO_4/NaH_2PO_4 buffer. Standards were prepared by the addition of known quantities of acivicin to drug-free plasma or CSF at the same dilution as the samples. Aliquots of samples and standards were applied to paper discs (Schleicher and Schuell Co, Keene, NH) on agar trays seeded with the microorganism. A complete set of six standards (ranging from 0.059 to 0.44 μg/ml) was applied to each tray; each sample and standard was applied in triplicate to each tray. Trays were incubated at 37°C for 15–18 hrs, and the zones of inhibition were measured to the nearest millimeter. Standard curves were constructed by plotting the log concentration of the standards versus zone size. Standard curves in plasma diluted 1:200 to 1:30 and in CSF at all dilutions were straight lines; a statistical least-squares linear regression program for a programmable calculator was used to fit equations to the lines and interpolate the sample values. Standard curves in plasma at lower dilutions were curvilinear and sample values were visually interpolated. Most samples were assayed at two dilutions and the results were averaged. Samples with concentrations falling outside of the standard curve range were reassayed at different dilutions.

Bioautographic Studies

Bioautographic studies were performed to determine the specificity of the microbiologic assay. Aliquots of selected plasma and CSF specimens containing previously assayed amounts of acivicin bioequivalents were applied to plastic-backed thin-layer chromatography sheets (Baker-flex IB-F flexible sheets, J. T. Baker Chemical Co, Phillipsburg, NJ) along with spiked samples containing known amounts of authentic acivicin. The volume applied was < 10 μl. After sample application, the plates were dried in a stream of warm air and developed in tetrahydrofuran:acetone:water (5:3:2), methylethyl ketone:acetone:water (2:1:1), acetone:water (8:2), or isopropanol:28.5% ammonium hydroxide:water (7:1:2). Following development, the sheets were dried in a stream of warm air for 3 hrs. The silica gel surface of the sheets was then carefully placed in contact with agar trays seeded with *B. subtilis* (synthetic medium) for 30 mins. The sheets were peeled off and discarded, and the trays were incubated. The resulting zones of growth inhibition for samples were then used to calculate R_f values, which were compared to those of acivicin standards. Only one zone of inhibition per sample was observed in all bioautographic studies, and in each case the R_f of the material corresponded to that of intact acivicin. Therefore, all pharmacokinetic calculations were done with the assumption that only intact acivicin was measured by the assay.

Pharmacokinetic Modeling

To define the dynamic relationship between the CSF and plasma concentrations, compartmental

pharmacokinetic modeling was carried out on the data. The triexponential equations for the time course of drug concentrations in the three-compartment open model for drug disposition (17) were curve-fitted simultaneously to the plasma and CSF concentrations of each monkey (fig 1).

In this model, the central compartment consists of plasma and rapidly perfused tissues and the peripheral compartment consists of poorly perfused tissues. The "brain" compartment is defined as containing CSF and brain extracellular and intracellular spaces, all of which are assumed to be in rapid equilibrium. Elimination by metabolism and excretion is assumed to occur only from the central compartment. Metabolism of acivicin within the brain is certainly possible, but the data are not sufficient to allow incorporation of this aspect into the pharmacokinetic model.

The equations describing the three-compartment model and additional calculated parameters are given in the Appendix. The coefficients, exponents, and compartmental volumes in the integrated equations were estimated by simultaneous iterative curve fitting of the plasma and CSF concentrations using the nonlinear least-squares parameter estimation program, NONLIN, on an IBM 370/158 digital computer (18). Initial estimates of fitted parameters were obtained by manual feathering techniques (17). To minimize the difference in magnitude between the plasma and CSF concentrations for computer fitting, the data were weighted using the factor, $1/yi^2$. That is, the following function was minimized:

$$\sum_{i=1}^{N} 1/y_i^2\,(yi_{obs}-yi_{calc})^2,$$

where yi_{obs} is the observed value of the ith measurement, yi_{calc} is the calculated or fitted value of the ith measurement, and N is the number of data points. This weighting approach is statistically valid because all plasma and CSF samples were diluted to the same narrow concentration range prior to assay (19,20).

Attempts were made to fit the three-compartment model, with all rate constants and volumes (including V_3) allowed to vary and with V_3 fixed at the approximate anatomic volume of the brain in a monkey, 0.03 liter/kg (21). Goodness of fit was determined from correlation coefficients (r^2), residual sums of squares, standard deviations of estimated parameters, and visual inspection of plots of the predicted and observed concentrations versus time.

RESULTS AND DISCUSSION

No adverse effects of acivicin administration at either dose were observed upon daily physical examination or in the hematology and blood chemistry studies. Although no previous single-dose toxicity studies had been conducted in monkeys, 2.6 mg/kg/day on the daily × 5 schedule was determined to be a lethal dose (6).

Figure 2 shows plasma and CSF concentrations of acivicin in monkeys receiving single 4- or 20-mg/kg doses iv. Plasma concentrations declined in a multiexponential fashion, with a terminal (elimination phase) half-life of 3–4 hrs. The initial (distribution) phase in plasma appeared to be complete by approximately 1 hr after dosing. As indicated by the ratio of the areas under the plasma level curves (AUC_1) at the two doses (table 1), plasma concentrations were proportional to the doses administered. CSF concentrations also appeared to be proportional to the dose. CSF concentrations increased over a period of 2–2.5 hrs and then declined in parallel to plasma levels with CSF/plasma concentra-

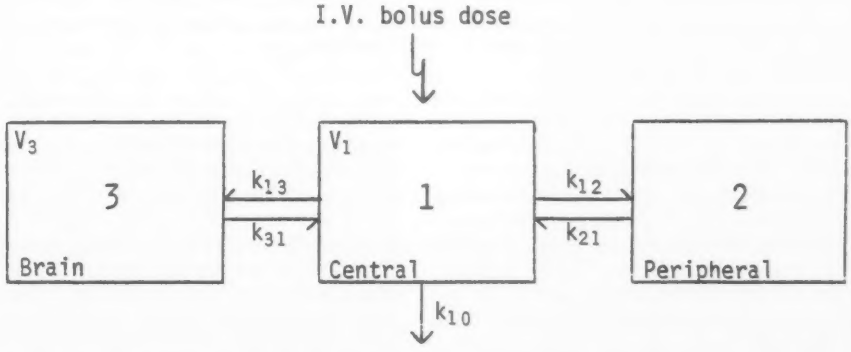

FIGURE 1.—The 3-compartment open model for acivicin disposition.

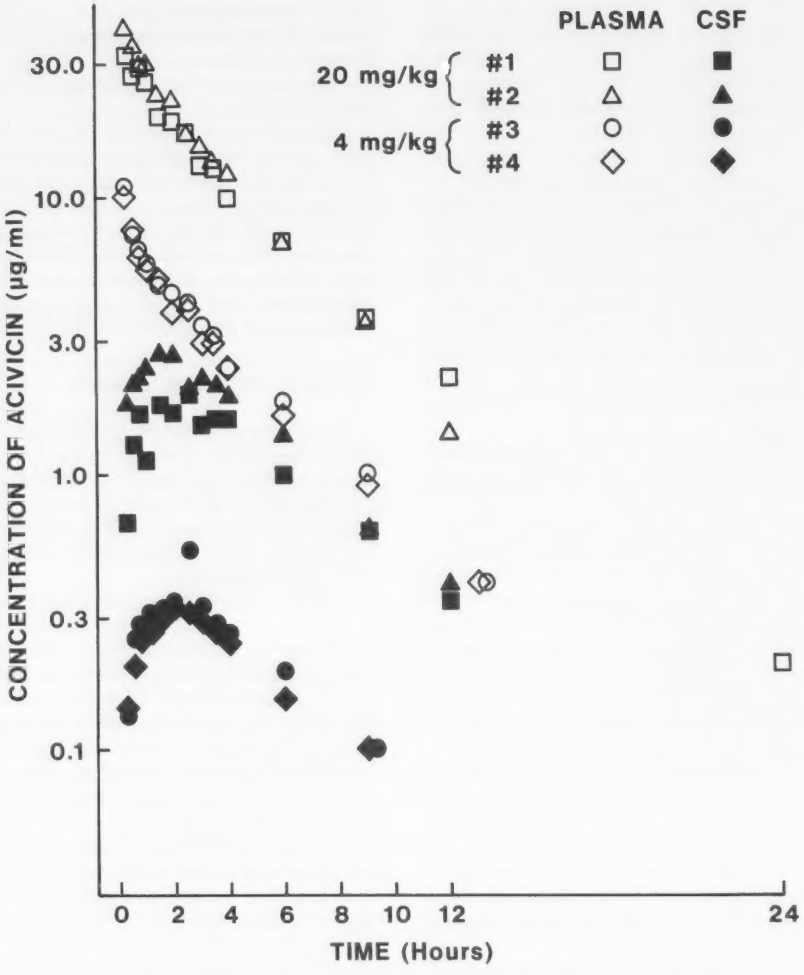

FIGURE 2.—Time course of plasma and CSF concentrations of acivicin following 20- and 4-mg/kg iv doses in 4 rhesus monkeys.

tion ratios of 0.10–0.17 in the postdistributive phase. CSF concentrations > 1.0 µg/ml were maintained for almost 6 hrs at the 20-mg/kg dose.

Free diffusion of acivicin, a water-soluble amino acid in the zwitterionic form at physiologic pH, across the blood-brain barrier was not expected to occur. The observed degree of uptake into CSF from blood can, therefore, be attributed to some type of transport process, as has been postulated for other neutral amino acids (22).

On initial examination, the data appeared to be suitable for description by a two-compartment open model for drug disposition. However, the initial rapid decline in plasma concentrations (distribution phase) was complete by approximately 1 hr after dosing, whereas CSF concentrations did not peak and begin to decrease in parallel with the plasma levels until 2–3 hrs. This feature of the data is inconsistent with the two-compartment model and implies that in addition to the partitioning into CSF, drug is distributing from plasma into a compartment kinetically distinct from that containing the CSF. Therefore, a three-compartment model is necessary to describe the data. Since CSF and brain are separated from

TABLE 1.—Fitted and derived pharmacokinetic parameters of 3-compartment model for iv administered acivicin in plasma and CSF of rhesus monkeys*

Parameters	Monkey No. 1	2	3	4	Mean	SD
Dose (mg/kg)	20	20	4	4		
Weight (kg)	5.4	6.1	7.6	8.6	6.9	1.4
P	0.43	0.35	0.65	0.58	0.50	0.14
A	0.0023	0.0052	0.00084	0.0023	0.0027	0.0018
B	0.57	0.65	0.35	0.41	0.50	0.14
P′	–0.0016	–0.0020	–0.0012	–0.0013	–0.0015	–0.00036
A′	–0.0039	–0.0069	–0.0051	–0.0039	–0.0050	–0.0014
B′	0.0055	0.0090	0.0063	0.0052	0.0065	0.0017
π (hr^{-1})	1.91	2.36	4.38	3.06	2.93	1.08
α (hr^{-1})	0.67	0.89	0.61	0.65	0.71	0.13
β (hr^{-1})	0.20	0.26	0.23	0.23	0.23	0.02
$t_{1/2_\pi}$ (hr)	0.36	0.29	0.16	0.23	0.26	0.09
$t_{1/2_\alpha}$ (hr)	1.03	0.78	1.14	1.07	1.01	0.16
$t_{1/2_\beta}$ (hr)	3.47	2.67	3.01	3.01	3.04	0.33
V_1 (liters/kg)	0.47	0.39	0.20	0.27	0.33	0.12
V_B (liters/kg)	0.78	0.57	0.52	0.57	0.61	0.12
k_{10} (hr^{-1})	0.33	0.38	0.60	0.49	0.45	0.12
k_{12} (hr^{-1})	0.61	0.60	2.32	1.38	1.23	0.81
k_{21} (hr^{-1})	1.17	1.63	1.69	1.40	1.47	0.24
k_{13} (hr^{-1})	0.0046	0.0088	0.0024	0.0066	0.0056	0.0027
k_{31} (hr^{-1})	0.67	0.88	0.61	0.65	0.70	0.12
AUC_1 (mg-hr/liter)	129.4	136.5	32.6	30.5		
AUC_3 (mg-hr/liter)	13.8	17.1	2.5	2.2		
Cl_B (ml/hr)	837	904	912	1138	948	131
Cl_{31} (ml/hr)	109	161	139	168	144	27

*Volume of distribution of brain compartment, V_3, was fixed at the estimated anatomic volume of CSF in a monkey, 0.03 liter/kg, for computer fitting of data from all animals. All other constants and volumes were allowed to vary or "float." For definition of parameters, *see* the Appendix.

the general circulation and other "peripheral" tissues by the blood-brain barrier, the three-compartment model is also appropriate from an anatomic standpoint. Although the fitting program converged to a solution, large standard deviations of estimated parameters resulted when all rate constants and volumes (including that of the brain compartment) were allowed to vary or "float" in the fitting program. In subsequent fitting, V_3 was fixed at the approximate anatomic brain volume of a monkey, 0.03 liter/kg (21). This assumption was supported by the computer estimates of V_3 obtained for the four monkeys when all constants and volumes were varied: 0.030, 0.031, 0.011, and 0.037 liter/kg.

The plasma concentration-time course data do not have the triphasic appearance typical of data that are appropriately described by a three-compartment model, apparently because the volume of the compartment containing CSF was quite low compared to that of the central compartment; ie, the actual amount of drug in the "brain" compartment at any time was very small relative to that in the rest of the system, and there was no detectable effect of that particular distribution process on the kinetics in plasma. Fitting the plasma and CSF data simultaneously to a three-compartment model allowed reasonable estimates of the central-CSF distribution constant, π, to be made from the CSF data (which contained insufficient information on the central-peripheral distribution) and of the central-peripheral distribution constant, α, to be made from the plasma data (which contained insufficient information on the plasma-CSF distribution).

Table 1 summarizes the fitted and derived parameters of the three-compartment model describing acivicin kinetics in plasma and CSF of monkeys. Figure 3 shows plots of the predicted and observed plasma and CSF concentrations versus time for the two monkeys having the best and worst fits of the four sets of data analyzed. There was excellent agreement between the predicted and observed curves as demonstrated by the visual fit, the r^2 values, and the standard deviations of estimated pa-

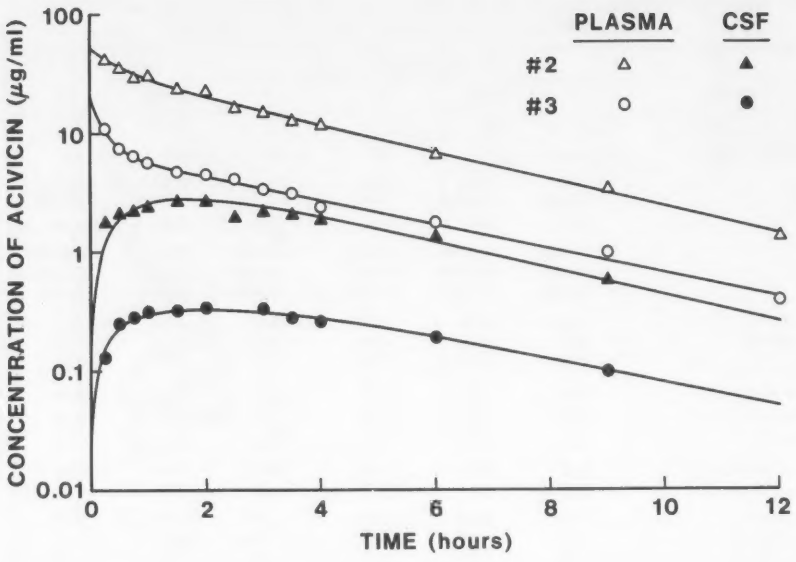

FIGURE 3.—Predicted and observed plasma and CSF concentrations of acivicin in 2 monkeys having the best (monkey No. 3) and worst (monkey No. 2) fits of the 3-compartment pharmacokinetic model to the 4 sets of data in fig 1. Lines were generated from equations describing the 3-compartment model using computer-estimated "best-fit" parameters for each animal (table 1).

rameters. The magnitude of k_{13} relative to the other transfer constants reflects the small mass of material entering the CSF compartment at any time. There appeared to be some dose dependency of the parameters V_1 and k_{12}.

The calculated value of the CSF-to-plasma intercompartmental clearance, $Cl_{31} = k_{31} \cdot V_3$, was dependent on the assumption that acivicin was distributed throughout the total brain volume and that all parts of the brain were in rapid equilibrium. As such, the absolute magnitude of Cl_{31}, 144 ml/hr, may not be directly comparable to measured CSF clearances of several radiolabeled endogenous amino acids determined by ventriculocisternal perfusions in cats (23,24), which were 2–3 ml/hr, since distribution of the amino acids beyond the CSF into brain tissue was low. However, the magnitude of Cl_{31} relative to the estimated CSF flow rate of 4–6 ml/hr in the rhesus monkey (25) indicates that acivicin was cleared from CSF at a much faster rate than would be expected from bulk flow alone. Therefore, acivicin was apparently rapidly transported out of the CSF. Metabolism of acivicin within the brain could also contribute to its rapid clearance, but there were not sufficient data to allow estimation of a brain metabolism constant in the pharmacokinetic model.

Most substances in CSF are freely available to brain parenchyma (13). Acivicin concentrations in whole-brain homogenate were previously measured as a function of time in mice and dogs and found to be from 20% to 100% of simultaneous plasma concentrations (9; J. P. McGovren, unpublished data). It is not known at this time whether the CNS effects observed in humans receiving iv administered acivicin are due to the intact molecule or to a metabolite, but the data suggest that acivicin itself could be available to elicit such effects. Whether these concentrations of acivicin in human CSF would result in brain levels that would be cytotoxic to a malignant growth is also not known at this time, but clinical evaluation of the agent against CNS malignancies and metastases should be considered.

APPENDIX

Gibaldi and Perrier (17) give the integrated equation describing the drug concentration in the three-compartment open pharmacokinetic model (C_1) as:

$$C_1 = \frac{X_0}{V_1}\left[Pe^{-\pi t} + Ae^{-\alpha t} + Be^{-\beta t}\right]$$

where

$$P = \frac{(k_{21}-\pi)(k_{31}-\pi)}{(\pi-\alpha)(\pi-\beta)}$$

$$A = \frac{(k_{21}-\alpha)(\alpha-k_{31})}{(\pi-\alpha)(\pi-\beta)}$$

$$B = \frac{(k_{21}-\beta)(k_{31}-\beta)}{(\alpha-\beta)(\pi-\beta)}$$

The equation for the drug concentration in the CSF compartment (C_3) was derived using the Laplace transform method described by Gibaldi and Perrier (17):

$$C_3 = \frac{X_0}{V_3}\left[P'e^{-\pi t} + A'e^{-\alpha t} + B'e^{-\beta t}\right]$$

where

$$P' = \frac{k_{13}(k_{21}-\pi)}{(\pi-\alpha)(\pi-\beta)}$$

$$A' = \frac{k_{13}(k_{21}-\alpha)}{(\pi-\alpha)(\beta-\alpha)}$$

$$B' = \frac{k_{13}(k_{21}-\beta)}{(\alpha-\beta)(\pi-\beta)}$$

and

$$k_{31} = 1/2\left[-b-\sqrt{b^2-4ac}\right]$$

$$k_{13} = 1/2\left[-b+\sqrt{b^2-4ac}\right]$$

where

$$a = 1$$

$$b = -\frac{\pi B+\pi A+\beta P+\beta A+\alpha P+\alpha B}{P+A+B}$$

$$c = \frac{\alpha\pi B+\pi\beta A+\alpha\beta P}{P+A+B}$$

$$k_{10} = \frac{\alpha\beta\pi}{k_{21}k_{31}}$$

$$k_{12} = \frac{(\alpha\beta+\alpha\pi+\beta\pi)-k_{21}(\alpha+\beta+\pi)-k_{10}k_{31}+k_{21}^2}{k_{31}-k_{21}}$$

$$k_{21} = \alpha+\beta+\pi-(k_{10}+k_{12}+k_{21}+k_{31}).$$

C_n is the concentration of acivicin in compartment n; t is time; P, A, B, P′, A′, and B′ are coefficients; and α, β, and π are hybrid rate constants describing the concentration-time curves; X_0 is the dose; k_{10} is the elimination rate constant (sum of individual rate constants for all routes of elimination); k_{12}, k_{21}, k_{13}, and k_{31} are intercompartmental transfer rate constants; and V_1 and V_3 are the apparent distribution volumes of the central and CSF compartments.

Additional quantities calculated from the fitted parameters were:

$$V_B = \frac{X_0}{\beta\int_0^\infty C_1 dt} = \frac{V_1 k_{10}}{\beta}$$

$$AUC_1 = \int_0^\infty C_1 dt = \frac{X_0}{V_1}\left(\frac{P}{\pi}+\frac{A}{\alpha}+\frac{B}{\beta}\right)$$

$$AUC_3 = \int_0^\infty C_3 dt = \frac{X_0}{V_3}\left(\frac{P'}{\pi}+\frac{A'}{\alpha}+\frac{B'}{\beta}\right)$$

$$Cl_B = V_B \cdot \beta = V_1 \cdot K_{10}$$

$$Cl_{31} = V_3 \cdot k_{31},$$

where V_B is the apparent volume of distribution relating the plasma concentration to the total amount of drug in the body during the postdistribution phase; AUC_1 and AUC_3 are the total areas under the plasma and CSF concentration-time curves; Cl_B is the total body clearance of acivicin by metabolism and excretion; and Cl_{31} is the drug clearance from the CSF compartment.

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Studies on the *O*-Demethylation of Misonidazole by Rat Liver Microsomes[1]

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SUMMARY

The role of rat liver microsomes in the *O*-demethylation of misonidazole to desmethylmisonidazole was studied. The rate of the microsomal-dependent formation of desmethylmisonidazole was linear up to a protein concentration of 2 mg/ml and over a 10-minute interval. The metabolism was optimal in a system comprised of microsomes, O_2, and NADPH. Metabolism in incubation mixtures continuously flushed with N_2 was inhibited by 78%. The *O*-demethylase activity was competitively inhibited by the addition of SKF 525-A, with a Ki of approximately 1×10^{-5} M. The Km and Vmax values of normal microsomes were 1.87 ± 0.30 mM and 413 ± 14 pmols/minute/mg of microsomal protein, respectively. Pretreatment of rats with phenobarbital for 7 days prior to preparation of the microsomes resulted in no significant change in the Km, but the Vmax was considerably increased to 1033 ± 203 pmols/minute/mg of microsomal protein. The results demonstrate that the *O*-demethylation of misonidazole is mediated by cytochrome P-450.

[Cancer Treat Rep 66:1343–1347, 1982]

Misonidazole (fig 1), a hypoxic cell radiosensitizer presently undergoing clinical trials, is known to be extensively metabolized in rodents and man (1). The primary metabolite identified to date is desmethylmisonidazole and is formed by *O*-demethylation (1). Desmethylmisonidazole has been shown to be effective as a radiation sensitizer in vitro (2) and has been entered into phase I clinical trials (3). During clinical trials, misonidazole was observed to be associated with neurotoxicity (4), the incidence of which was lessened by concomitant therapy with inducers of the hepatic drug-metabolizing enzymes (5,6). In vivo studies in the mouse, the dog, and man demonstrated that pretreatment with known microsomal enzyme inducers increased both the plasma clearance of misonidazole and the percent of the dose excreted as the demethylated metabolite (7–9). No previous attempt has been made to study the conversion of misonidazole to the demethylated metabolite in the in vitro system. Therefore, the present studies of the *O*-demethylation of misonidazole to desmethylmisonidazole by liver microsomes prepared from normal and phenobarbital (PB)-pretreated rats were undertaken to characterize this metabolic reaction.

MATERIALS AND METHODS

Misonidazole was obtained from the National Cancer Institute (Bethesda, MD). Materials used in this study were: glucose-6-phosphate, glucose-6-phosphate dehydrogenase, NADH, and NADPH (Boehringer Mannheim Biochemicals, Indianapolis, IN); and phenobarbital sodium (Fisher Scientific Co, Fair Lawn, NJ). All other chemicals were of analytic reagent grade.

Male Sprague-Dawley rats (175–225 g, Taconic Farms, Germantown, NY) maintained on a diet of water and Purina Rat Chow (Ralston Purina Co,

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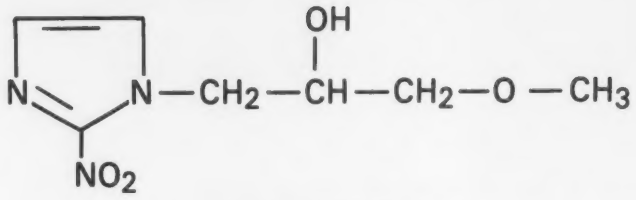
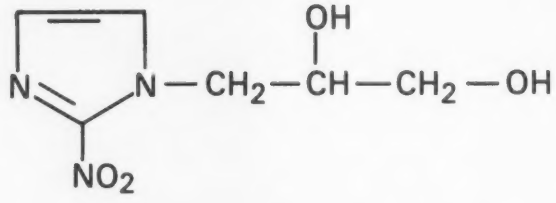


MISONIDAZOLE



DESMETHYLMISONIDAZOLE

FIGURE 1.—Structures of misonidazole and desmethylmisonidazole.

St. Louis, MO) were killed by decapitation. For the induction studies, rats were pretreated daily for 7 days with PB sodium at a dose of 75 mg/kg ip or saline and were killed by decapitation 24 hours after the last dose. The livers were removed, homogenized in ice-cold 0.1 M sodium phosphate buffer (pH 7.4), and subjected to centrifugal fractionation. The resulting microsomal pellet was washed once and resuspended. The microsomal protein concentrations were determined by the method of Lowry et al (10) using bovine serum albumin as the standard. The formation of desmethylmisonidazole was measured in 0.5 ml of incubation mixtures consisting of 1 mg of both NADH and NADPH, 100 mM sodium phosphate buffer (pH 7.4), 1 mg of microsomal protein, and concentrations of misonidazole ranging from 5×10^{-4} M to 5×10^{-3} M. The reactions were initiated by the addition of misonidazole and were incubated at 37°C in a Dubnoff metabolic shaker. At the appropriate times the reactions were stopped by the addition of 2 ml of ethyl acetate, followed by the addition of 0.85 nmol of the internal standard (1-[2-nitro-1-imidazolyl]-3-fluoro-2-propanol) in water, 50 µl of 0.1 M carbonate buffer (pH 10.0), and 200 µl of distilled water. The samples were mixed and centrifuged at 200 × *g* for 5 minutes in a Beckman model J-6B centrifuge. The resulting top layer was removed and reduced to dryness under nitrogen. The precipitate was reconstituted with 60 µl of 19% methanol in water and analyzed by the high-pressure liquid chromatography method of Workman et al (11).

RESULTS AND DISCUSSION

The results in figure 2 demonstrate that the metabolism of misonidazole to desmethylmisonidazole was linear with respect to microsomal protein concentrations up to 2 mg/ml and to incubation periods up to 10 minutes. The metabolism was optimal in a system composed of microsomes, O_2, and NADPH (table 1). When the incubation mixtures were continuously flushed with N_2, which removed the O_2 required to form the metabolite oxidatively, the conversion of misonidazole to the demethylated metabolite was inhibited by 78%. The use of NADH as the sole electron donor decreased the *O*-demethylase activity by 90% as compared to controls with NADPH alone.

The kinetic parameters for the metabolism of misonidazole were determined after incubation of misonidazole concentrations from 5×10^{-4} M to 5×10^{-3} M with liver microsomes prepared from normal

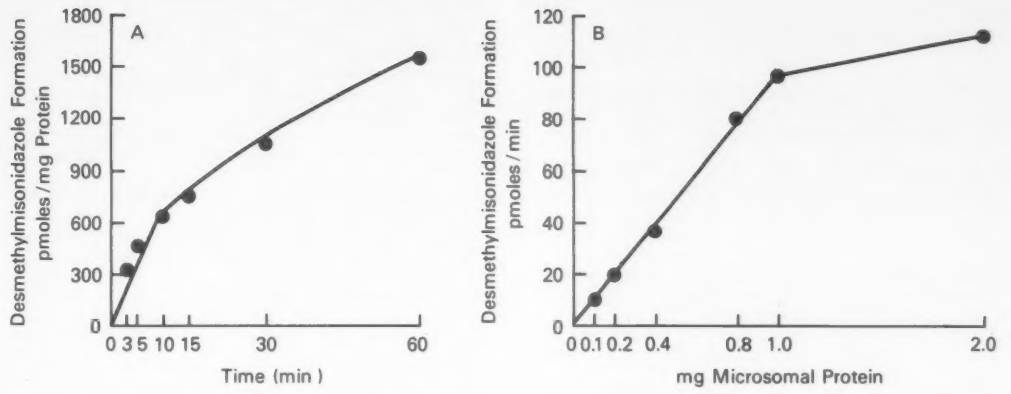


FIGURE 2.—In vitro metabolism of misonidazole to desmethylmisonidazole by rat liver microsomes was measured in 0.5 ml of incubation mixtures. A: formation of desmethylmisonidazole was measured at varying time points as described in the text, and incubation mixtures contained 1 mg of microsomal protein. B: linearity of metabolism was observed after 10 mins of incubation at varying concentrations of rat liver microsomal protein. Values = mean of duplicate determinations of 2 or 3 experiments.

and PB-pretreated rats. The values were fit directly to the following Michaelis-Menten equation:

$$V = \frac{Vmax\ [S]}{Km + [S]}$$

using the MLAB program on a PDP-10 computer (12). The apparent Km and Vmax values for normal microsomes were 1.87 ± 0.30 mM and 413 ± 14 pmols/minute/mg of protein, respectively. Pretreatment of rats with PB, a known inducer of hepatic microsomal enzymes (13), prior to preparation of the microsomes resulted in a comparable apparent Km, 1.70 ± 0.52 mM, while the Vmax was increased 2.5-fold to 1033 ± 203 pmols/minute/mg of protein. The Km and Vmax values are the mean ± SE of duplicate determinations of 4–5 experiments. Each incubation mixture of 0.5 ml contained 1 mg of microsomal protein, and the length of incubation was 10 minutes. In figure 3 the actual values are represented by the symbols, while the solid lines are a fit to the MLAB-derived Km and Vmax values.

TABLE 1.—Effect of incubation conditions on the *O*-demethylation of misonidazole*

Incubation condition	*O*-demethylation of misonidazole (pmols/10 mins/mg of protein)†	% inhibition
Complete system	1380.3 ± 64.7	0
+ N_2	304.4 ± 64.7	77.9‡
+ NADH only	145.5 ± 12.3	89.5‡
+ NADPH only	1385.7 ± 54.0	0

*Complete system is described in the text. Flasks for N_2 incubations were flushed continuously.

†Values = mean ± SE of 3 determinations.

‡Significantly different ($P < 0.05$) from value for complete system.

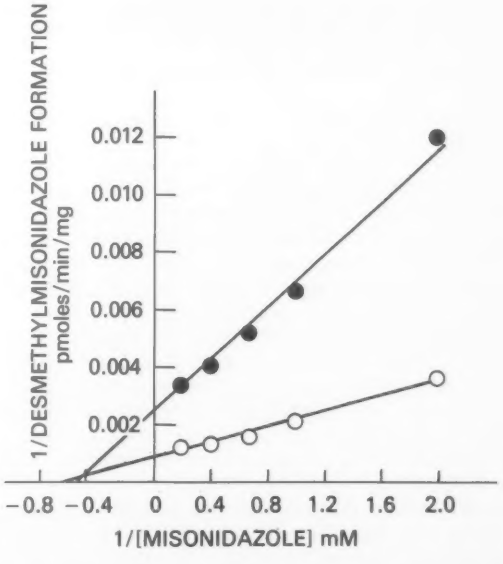


FIGURE 3.—Lineweaver-Burk plot of the *O*-demethylase activity of liver microsomes prepared from normal (●) and PB-pretreated (○) rats. Incubation mixtures contained 1 mg of microsomal protein and totaled 0.5 ml. Duration of incubation was 10 mins. Values = mean of duplicate determinations of 4 or 5 experiments.

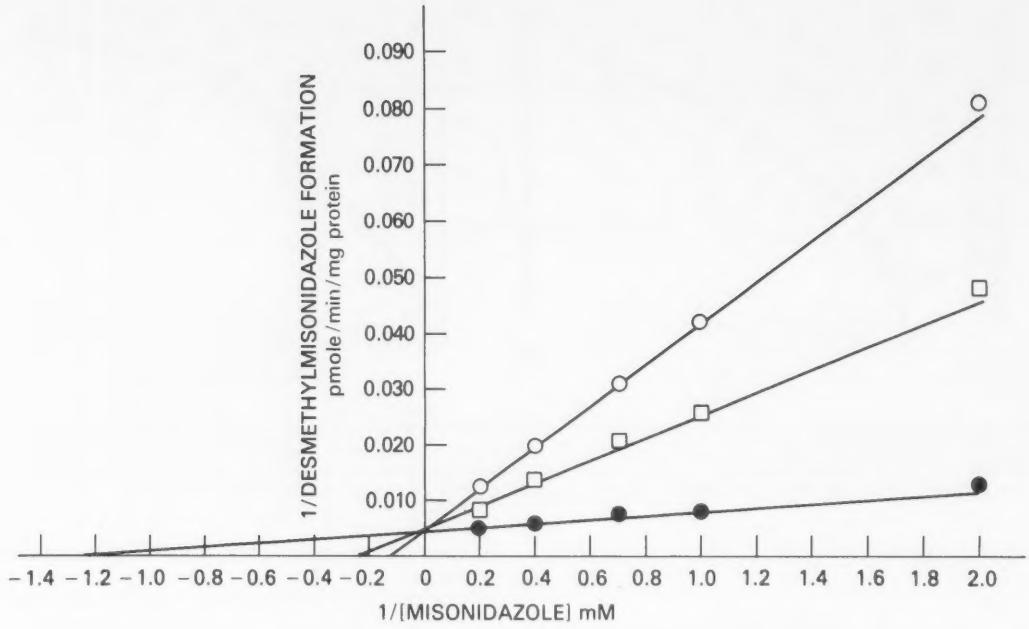


FIGURE 4.—Lineweaver-Burk plot of the inhibition of rat liver microsomal misonidazole *O*-demethylase activity by SKF 525-A. Misonidazole was incubated with 1 mg of rat liver microsomal protein in reaction mixtures totaling 0.5 ml for 10 mins, as described in the text, in the presence of 1×10^{-4} M (○) and 1×10^{-5} M (□) SKF 525-A or the SKF 525-A vehicle (●). Values = mean of duplicate determinations of 2 or 3 experiments.

The observed increase in the Vmax values compares favorably with the increase in the ratio of desmethylmisonidazole to misonidazole observed in rat urine after pretreatment with PB (14). The ratio increased approximately 3.0-fold from 0.8 in saline controls to 2.5 in PB-pretreated rats.

When SKF 525-A, an inhibitor of hepatic microsomal-mediated drug oxidations (15), was added at 10^{-4} and 10^{-5} M to varying concentrations of misonidazole, the resulting inhibition was observed to be of a competitive nature by a plot of the reciprocal values (fig 4). This suggested that misonidazole binds specifically to cytochrome P-450, as SKF 525-A has been shown to interact selectively with cytochrome P-450 (16). The Ki was determined to be approximately 1×10^{-5} M by a direct fit of the data using the MLAB program on a PDP-10 computer to the following equation:

$$V = \frac{Vmax\,[S]}{Km\left(1 + \frac{[I]}{Ki}\right) + [S]}.$$

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Ganglioneuropathy in Rabbits and a Rhesus Monkey Due to High Cumulative Doses of Doxorubicin[1,2]

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SUMMARY

The effects of long-term administration of doxorubicin in adult rabbits and in a rhesus monkey were studied. Eleven rabbits were given cumulative doses of 2–24 mg/kg over a period of 5–28 weeks. The monkey received a total of 20 mg/kg during a 10-month period. One rabbit exhibited hind leg paresis, but all of the other animals in the study remained clinically free of neurologic signs. Rabbits given < 12 mg/kg of doxorubicin had only mild degenerative changes in dorsal roots and a few necrotic neurons in the dorsal root ganglia, but these lesions were much more severe in all rabbits when a cumulative dose > 16 mg/kg was given. The monkey also had severe ganglioneuropathy, suggesting that primates may also be susceptible to this toxic effect of doxorubicin. Although neurotoxicity has not been observed with the dose schedules of doxorubicin used in clinical practice, the tendency of this drug to damage both postmitotic neural and cardiac cells may provide added understanding of the clinically important doxorubicin cardiomyopathy.

[Cancer Treat Rep 66:1349–1355, 1982]

Doxorubicin is both an effective anticancer chemotherapeutic agent and a potent toxin directed against mitotically active normal cells of the gut, hair follicles, and bone marrow (1). Myocardiopathic effects of doxorubicin therapy are well-known in man (2–4) and have been described in experimental animals (5). Paresis associated with necrosis of neurons in the dorsal root ganglia and other peripheral ganglia occurred in rats receiving a single dose of doxorubicin (6,7); no toxic effect on ganglion cells in any other species, including man, has been reported. Although the antimitotic activity of the drug can be explained by its ability to interfere with DNA and RNA synthesis (1), the biochemical basis of its toxic effects on postmitotic myocardial, cardiac ganglionic (8), and peripheral ganglionic cells is not understood.

This study was designed to determine if doxorubicin has neurotoxic effects when given in multiple doses to rabbits and to one rhesus monkey (*Macaca mulatta*). Evidence will be presented that both species develop ganglioneuropathy, the severity of which is dependent on the cumulative dose. Surprisingly, the lesions, although severe in animals receiving high cumulative doses, were clinically obvious in only one rabbit. The dose regimen and total cumulative dose received by the rabbits were similar to those used in rabbits in other experiments (5). They were the maximum tolerable doses that do not cause fatal myelosuppression. In that sense alone, they were somewhat comparable to those used in patients. The findings suggest that some patients treated with doxorubicin might also have subclinical neurohistologic changes.

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MATERIALS AND METHODS

The rabbits used in these experiments were male New Zealand White rabbits acquired from the Gloucester Rabbitry, Chepachet, RI. They weighed 2.0–2.9 kg at the start of the experiment. The animals were housed in separate stainless steel cages throughout the experiment and were fed 4–8 oz of Charles River Rabbit Formula each day. All animals were inspected daily; hematologic parameters were monitored weekly by cbc.

Eleven rabbits were given weekly injections of 2 mg/kg of doxorubicin administered by rapid iv push through a #22 scalp vein needle placed in the marginal vein of the ear. The cumulative dose varied from 2 to 24 mg/kg. Animals received the treatment until the designated cumulative dose was reached. This required > 12 weeks for animals receiving the highest dose because occasional weekly courses were omitted when the wbc count decreased to < 6000/mm^3 or the platelet count decreased to < 100,000/mm^3. Animals in good health at the time of the last course were permitted to live for 1 month afterwards; those in poor health were killed before that time. One high-dose rabbit was permitted to live for 14 weeks after the last dose to see if long-term sequelae would occur. One control rabbit was killed on the first day of the experiment. Two other controls received eight weekly courses of saline or saline plus dextrose, respectively.

At the conclusion of the experiment, all rabbits were anesthetized with pentobarbital sodium and killed by intracardiac perfusion of a mixture of 4% buffered formaldehyde and 1% glutaraldehyde (9). The central and peripheral nervous tissues were partially dissected and fixation was continued by immersion in the same fixative. One rabbit that developed posterior paresis was killed when moribund; tissues were fixed by immersion in 10% Formalin. For all rabbits, the brain, one trigeminal ganglion, spinal cord, and one or two lumbar dorsal root ganglia with associated dorsal root, ventral roots, and peripheral nerve were sampled and embedded in paraffin. Sections were stained with hematoxylin and eosin (H and E). To compare severity of lesions of different animals, replicate paraffin sections (6-μm thick) of lumbar dorsal roots, dorsal root ganglia, dorsal roots, ventral roots, and peripheral nerves were prepared and stained with H and E, Bodian, and luxol fast blue stains. Small samples of the left myocardium from some rabbits were dehydrated in graded solutions of alcohol and embedded in Epon. Semithick sections were stained with toluidine blue.

Two wild-caught mature female rhesus monkeys were acquired from the New England Regional Primate Research Center. They were housed in standard stainless steel cages and fed commercial chow supplemented with fruits and vegetables. These monkeys were initially included in a study designed to monitor cerebrospinal fluid drug levels, and as part of that study a plastic catheter attached to an Omaya reservoir was placed in the cisterna magna. One monkey was given five courses of doxorubicin at a dose of 4 mg/kg iv, 46, 38, 33, 22, and 12 weeks before death. In addition, the animal received a single dose of 10 g/m^2 of monobutyl methotrexate 72 hours before death. No other drugs were administered. The one other control monkey was given several courses of monobutyl methotrexate but no doxorubicin. The health of the monkeys was monitored by daily inspection and periodic blood cell counts. Post-mortem examinations were performed just after death. Tissues were fixed by immersion in 10% Formalin and processed as described above.

RESULTS

Results of the experiment in rabbits are summarized in table 1. Rabbits 1–9 were in good health throughout the studies. All recovered satisfactorily from marrow suppression after each course of doxorubicin. Of the five rabbits that received larger cumulative doses, one remained in excellent clinical condition throughout the 14 weeks following cessation of treatment and was electively killed. Three others were killed while still receiving weekly injections because they were anorectic and listless. Rabbit 13 was killed when moribund. This animal had been unable to adduct its hind legs and therefore had been unable to stand for 1 week before death. However, it was the only rabbit to develop neurologic signs.

For most of the experimental period the monkey was thin but maintained her appetite. The animal's head tilted slightly to the left following catheter placement in the cisterna magna, but no paresis, paralysis, or other neurologic abnormalities were noted. She was not specifically examined for more subtle neurologic deficits. The monobutyl methotrexate administered 72 hours before death precipitated acute renal failure and heart failure. BUN increased from 19 to 82 mg/100 ml, creatinine increased from 0.6 to 4.8 mg/100 ml, and acute-onset edema and ascites were apparent.

At necropsy, rabbits given lower doses of doxorubicin had no gross lesions. Those given cumulative doses of 24 mg/kg had hydropericardium and dilated hearts. The monkey given doxorubicin had extensive serous effusions in the body cavities and edema of extremities.

Microscopic examination was restricted to the nervous system and myocardium in the rabbits. The

TABLE 1.—Dose regimens used in the study of doxorubicin-induced ganglioneuropathy in rabbits

Rabbit No.	No. of wks of therapy (2 mg/kg)*	Time elapsed between last dose and death (wks)	Cumulative dose (mg/kg)	Severity of dorsal root lesions†	Severity of myocardial lesions‡
1§	0	—	—	0	0
2	1	4.5	2	1+	NT
3	2	4.5	4	1+	NT
4	3	4.5	6	2+	0
5	4	4	8	2+	1+
6	6	4	12	2+	NT
7	8	4	16	3+	2+
8¶	8	4	—	0	0
9**	8	4	—	0	0
10	11	0	20	3+	NT
11	14	0	24	3+	3+
12	13	2	24	3+	3+
13	16	1	24	3+	1+††
14	13	15	24	3+	3+††

*Some weekly doses were omitted because of myelosuppression.

†0 = no lesions; 1+ = occasional vacuoles/several 10× fields; 2+ = a few vacuoles/field; and 3+ = many vacuoles/field.

‡0 = no lesions; 1+ = rare vacuolated myocardial fiber (vf)/several fields; 2+ = 1 vf/high power field (hpf); and 3+ = many vf/hpf. NT = not tested.

§Untreated control.

¶Saline control.

**Saline and dextrose control.

††Scored independently by Billingham et al (2).

nature and severity of lesions varied considerably from rabbit to rabbit (table 1). Control rabbits had no lesions in the peripheral or central nervous systems (fig 1). In rabbits that were killed 1 month after 2–4 mg/kg of doxorubicin, the dorsal roots were normal except for occasional vacuoles containing myelin and axonal debris and lipid-laden macrophages (fig 2). All other portions of the central and peripheral nervous systems were normal. The numbers of vacuoles increased somewhat in rabbits given 6–12 mg/kg, but no more than a few per field of the 10× objective microscopic lens were present. The dorsal root ganglia were generally normal in these, but occasional clusters of satellite cells or neuronophages presumably marked the sites of necrotic ganglion cells.

The rabbits that received cumulative doses ≥ 16 mg/kg had many more vacuoles in the dorsal roots than did the rabbits receiving smaller doses (fig 3). The dorsal root ganglia also had many more clusters of neuronophages (fig 4). Rare neurons had such abnormal features as pyknotic nuclei, cytoplasmic vacuoles (fig 5), and amorphous pale eosinophilic cytoplasmic inclusions (fig 6). The degree of severity of lesions varied in roots and ganglia of rabbits with higher doses. However, at doses > 16 mg/kg no clear-cut dose-response relationship was evident. Whether this reflected variable response on the part of the individual rabbit or of the roots and ganglia was not fully studied here. However, two lumbar ganglia dissected from the rabbit that received a cumulative dose of 12 mg/kg were virtually identical in severity. This was also true for one of the rabbits that received 24 mg/kg.

All ventral roots were completely normal (fig 7). Peripheral nerves distal to the dorsal root ganglia had a few vacuoles. The sciatic nerves studied had no lesions visible by light microscopy. The spinal cords from rabbits with > 16 mg/kg of doxorubicin had vacuoles with shrunken macrophages in the dorsal column and less often in the lateral columns. Ventral horn cells were normal. Trigeminal ganglia examined were similar to the dorsal root ganglia. Vacuoles with gitter cells were present in the spinal trigeminal tract of rabbits that received the larger doses.

The myocardia from the three control rabbits and from the rabbit that received 6 mg/kg of doxorubicin were histologically normal (table 1). By searching many hpfs of the sections from the rabbit that received 8 mg/kg, a rare vacuolated myocardial fiber was found. One such fiber per hpf was present in the section from the rabbit that received 16 mg/kg. Many fibers were vacuolated in three of the four rabbit hearts receiving cumulative doses > 20 mg/kg. Three of the six rabbits with severe neural damage also had severe cardiac damage. In contrast, two of the five rabbits with milder neural damage

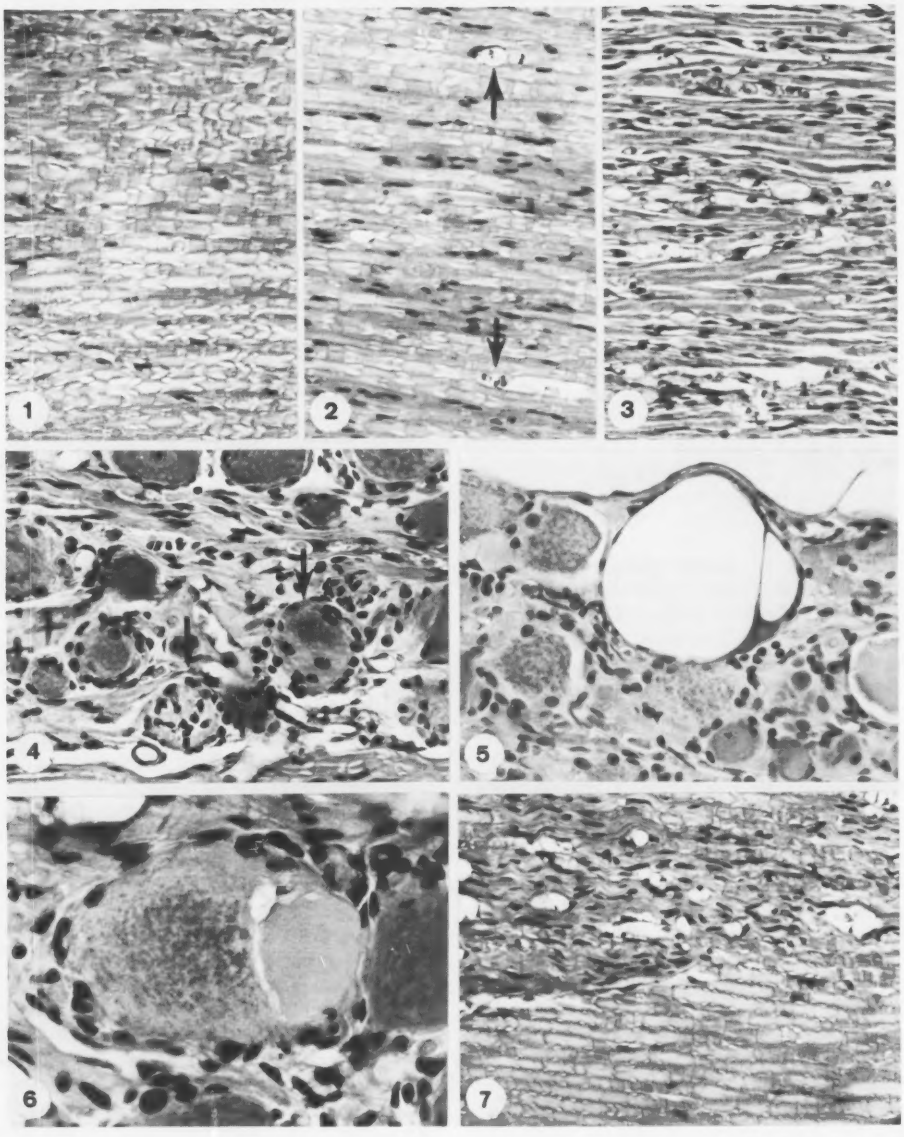
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FIGURE 1.—Longitudinal section of lumbar dorsal root of rabbit 8 that received only saline injection (H and E stain, × 135).

FIGURE 2.—Dorsal root from rabbit 3 that received 2 courses of doxorubicin. Few vacuoles containing pyknotic macrophages and cell debris are present (arrows) (H and E stain, × 135).

FIGURE 3.—Dorsal root from rabbit 7 that received 8 courses of doxorubicin. Numerous vacuoles are present (H and E stain, × 135).

FIGURE 4.—Dorsal root ganglion from rabbit 7. Several ganglionic cells are undergoing neuronophagia (arrows) (H and E stain, × 200).

FIGURE 5.—Ganglionic cell from rabbit 13. Cytoplasm is markedly vacuolated and has a peripherally displaced nucleus (H and E stain, × 350).

FIGURE 6.—Ganglionic cell from rabbit 10 has a large pale eosinophilic inclusion in the cytoplasm (H and E stain, × 540).

FIGURE 7.—Dorsal root (above) and ventral root (below) of rabbit 13. Dorsal root has many vacuoles. Relative loss of axons and myelin is evidenced by the much greater packing density of Schwann cell nuclei in the dorsal root as compared with the ventral root (H and E stain, × 135).

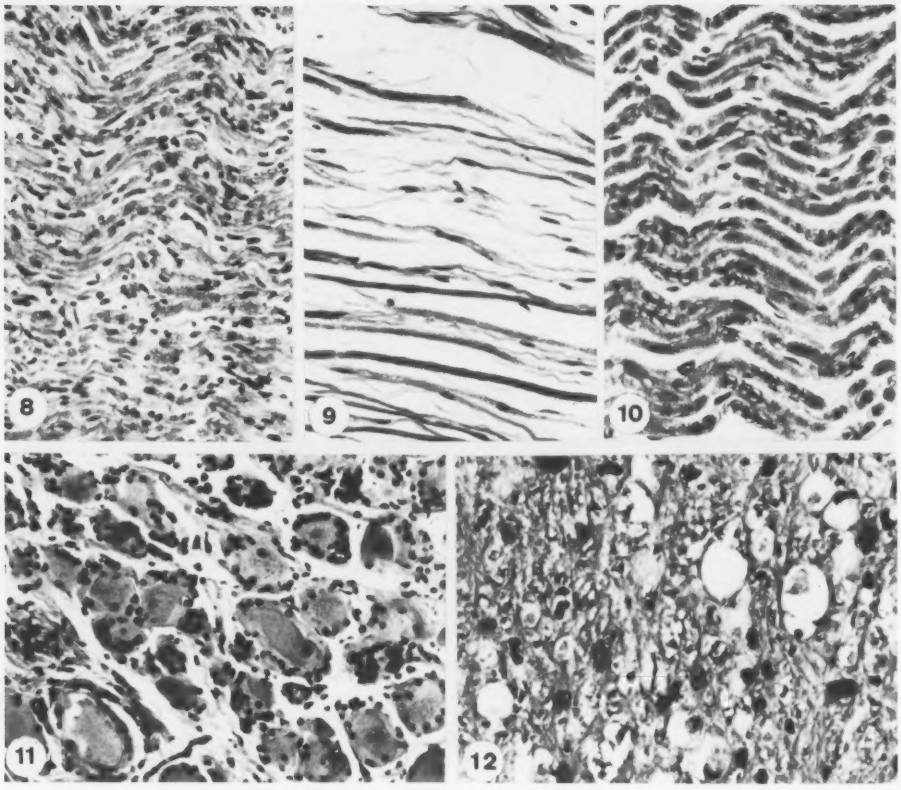

FIGURE 8.—Dorsal root of doxorubicin-treated monkey with loss of axons and myelin (H and E stain, × 135).

FIGURE 9.—Another dorsal root from the same monkey with marked loss of large fibers (H and E stain, × 135).

FIGURE 10.—Ventral root from doxorubicin-treated monkey with normal amounts of myelin and numbers of large myelinated axons (H and E stain, × 135).

FIGURE 11.—Dorsal root ganglion from a monkey that received multiple doses of doxorubicin. Many ganglionic cells are undergoing neuronophagia (H and E stain, × 135).

FIGURE 12.—Cross-section of lumbar spinal cord in the area of the fasciculus gracilis from the doxorubicin-treated monkey. Large vacuoles contain cell debris and macrophages (H and E stain, × 135).

had 0–1+ heart changes; three of these hearts were not examined histologically. Thus, there was a similar dose-toxicity pattern observed in both neural and cardiac tissues.

The monkey given doxorubicin had the most severely affected dorsal roots and ganglia of any animal studied. Some portions of the dorsal roots had numerous vacuoles; others exhibited varying degrees of nerve fiber and myelin loss (figs 8 and 9) as compared to ventral roots (fig 10). Numerous ganglionic cells were replaced by clusters of neuronophages (fig 11). Vacuolated neurons and eosinophilic necrotic neurons were also rarely observed as in the rabbits.

Dorsal columns throughout the spinal cord had numerous vacuoles containing cell debris and macrophages (fig 12). The brain was generally normal. The spinal trigeminal tract on the side contralateral to the catheter was also vacuolated. A glial scar was present in the deep left cerebellar white matter which marked the site of the catheter tip and the fourth ventricle was mildly dilated. There was severe myocardial degeneration and fibrosis in the heart of the monkey. The control monkey given methotrexate alone had no lesions in the brain, spinal cord, dorsal roots, ganglia, peripheral nerve, or myocardium.

DISCUSSION

The neuropathologic observations reported here are not substantially different from those described in doxorubicin-treated rats (6,7). As in rats, unipolar neurons of rabbit and monkey dorsal root ganglia and trigeminal ganglia underwent degeneration. The dose-response, time course, and clinical manifestation in rabbits and in the monkey differed considerably from those in rats, however. In rats, a single dose of 10 mg/kg causes paresis and widespread necrosis in dorsal root ganglia (6,7). In this study, successive weekly doses of 2 mg/kg caused only mild lesions in heart and nervous system at low cumulative doses. At cumulative doses > 16 mg/kg, lesions in both organ systems were more severe. Neurologic signs comparable to the paresis described in rats were present in only one high-dose rabbit. Whether other more subtle abnormalities, such as reduced nerve conduction time or impairment of conscious proprioception, were present was not considered in this study.

In view of the observations in the monkey given doxorubicin it is possible that humans treated with doxorubicin develop a similar ganglioneuropathy. The absence of overt clinical signs in the monkey suggests that such lesions might be clinically silent in man. The issue should be clarified through further experiments that include careful neurologic examination of affected monkeys. At the present time, the clinical significance of these neuropathologic observations in monkeys and rabbits is unknown. The greatest scientific importance of these findings may be in providing further insight into the unusual effects of doxorubicin on the postmitotic cell.

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Altered Hepatic Disposition of Doxorubicin in the Perfused Rat Liver at Hyperthermic Temperatures[1,2]

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SUMMARY

The perfused rat liver was used to study the effects of heat on hepatic functions and on the metabolism and biliary excretion of doxorubicin (Ad). Livers were perfused for 1 hour at 37°C and 42°C in the presence of biosynthetic substrates with or without Ad. Gluconeogenesis was decreased at both temperatures in the presence of Ad. Bile flow and excretion of Ad ceased at 42°C. Perfusate levels of Ad were not significantly different in the 1-hour perfusions. Livers were then perfused at 37°C and 41°C for 3 hours in the presence of 10 mM glucose and amino acids. The beta half-life of Ad measured as total fluorescence and as parent drug was prolonged at 41°C. The area under the plasma concentration-time curve was unchanged for parent drug but increased from 2010 μg/ml·min at 37°C to 4477 μg/ml·min at 41°C for total drug equivalents. Biliary excretion of total Ad equivalents was 7.39% ± 1.35% of the total dose at 37°C and 2.56% ± 2.08% of the total dose at 41°C ($P < 0.05$). Biliary excretion of Ad was 2.07% ± 0.37% of the total dose at 37°C and 1.31% ± 0.84% of the total dose at 41°C ($P < 0.05$). Hepatic tissue levels of total Ad equivalents were 288.7 ± 21.2 μg/g at 37°C and 322.7 ± 10.6 μg/g at 41°C ($P < 0.05$). Tissue levels of Ad were 57.5 ± 14 μg/g at 37°C and 23.0 ± 10.7 μg/g at 41°C ($P < 0.05$). Bile flow at the two temperatures decreased after 2 hours of perfusion. The greatest decrease in bile flow occurred during the third hour of perfusion at 41°C. The data indicate that at elevated temperatures there was a significant alteration in the pharmacokinetics of Ad in the perfused rat liver system. Since the liver plays a primary role in the metabolism and elimination of Ad, the observed changes might also occur during whole-body hyperthermia.

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The effects of hyperthermia and of chemotherapy on established tumors and on cells in culture were shown to enhance the cytotoxicity of one another (1). The interaction of doxorubicin (Ad) and hyperthermia has been of particular interest because of the clinical effectiveness of Ad in the treatment of a wide variety of human neoplasms. EMT-6 mammary sarcoma cells (2) and Chinese hamster cells (3) in culture showed increased sensitivity to Ad. Local heating of murine tumors, C3H mammary carcinoma (3), EMT-6 tumor, and KHT mammary carcinoma (4,5) enhanced the cytotoxic effects of systemically administered Ad but failed to enhance the antitumor effects. However, local tumor hyperthermia enhanced the effectiveness of systemically administered Ad against the 16C mouse mammary adenocarcinoma (6). No enhancement of Ad

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antitumor activity by whole-body hyperthermia could be demonstrated in the treatment of mice bearing P388 leukemia, mammary adenocarcinoma 16C, and colon carcinoma 38 (7).

There is a paucity of data on the effects of temperature on pharmacokinetics (8). In man, the proportion of salicylamide transformed to its glucuronide was decreased during pyrogen-induced fever (8). Plasma antipyrine half-life was prolonged during etiocholanolone-induced fever, probably due to impaired hepatic metabolism (8). Abnormal sulfobromophthalein retention times were found for all patients with pyrogen-induced fever (8). These results could be the direct effect of heat on hepatic metabolism or changes in renal and hepatic blood flow (9,10). Elevation of body temperature by means of a pump system in animals increased renal blood flow and glomerular filtration rates (9), but temperature increases due to heating the body surface were accompanied by a decline in hepatic and renal blood flow (10).

Hyperthermia significantly prolonged the half-life of antipyrine and the activation of cyclophosphamide in the perfused rat liver (11). A decrease in activity of microsomal suspensions was observed at temperatures > 40°C (12). However, hyperthermia produced minimal effects on the tissue distribution, metabolism, and excretion of Ad in the rabbit (13).

Although Ad may not be of use with whole-body hyperthermia, it could be used systemically with local hyperthermia or in regional perfusion such as in the liver. Therefore, these studies were undertaken to study the effects of hyperthermia and Ad on perfused liver functions and to observe any alterations in the metabolism or disposition of Ad in the isolated perfused liver system at elevated temperatures. Since the liver plays a primary role in the metabolism and elimination of Ad, study of the liver under hyperthermic conditions can also provide results relevant to treatment with whole-body hyperthermia.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley albino rats (King Rat, Madison, WI) weighing 200–250 g were fed laboratory chow ad libitum and fasted for 24–30 hours prior to liver perfusion.

Materials

The enzymes and cofactors necessary for the analysis of perfusate samples were obtained either from Boehringer Mannheim Biochemicals, Indianapolis, IN, or Sigma Chemical Co, St. Louis, MO. Bovine plasma albumin was purchased from Reheis Chemical Co, Phoenix, AZ. All other chemicals were of reagent grade. Ad was from Adria Laboratories, Inc, Columbus, OH.

Perfusate

The recirculating perfusate consisted of 150 ml of Krebs-Ringer bicarbonate solution containing 20% (vol/vol) washed outdated human erythrocytes and 2.5% (wt/vol) bovine plasma albumin, Cohn fraction V. During perfusions with an experimental time period of 3 hours, the perfusate also contained 10 mM glucose and amino acids equal to ten times the "normal" concentration of each amino acid in rat plasma. A membrane oxygenator (Sci-Med Life Systems, Inc, Minneapolis, MN) was employed for gassing the perfusate with O_2:CO_2 (95:5).

Perfusion Technique

The technique employed for in situ perfusion of the liver is that described by Collins and Skibba (14) for accurate and precise control of liver temperature. Thermocouple microprobes (Bailey Instrument Co, Saddle Brook, NJ) were used to monitor and control the liver, cabinet, and perfusate temperatures.

A perfusion period of 38 minutes was allowed for equilibration of the preparation. All substrate and drug additions were made immediately thereafter. The livers were perfused for an additional 60–180 minutes after initial sampling at 40 minutes (time zero of the experimental period), allowing 2 minutes for adequate mixing of the drug or substrates.

Analytic Methods

Perfusate samples were obtained at the specified intervals and protein-free filtrates were prepared from the perfusate samples with perchloric acid. Aliquots of protein-free filtrates were analyzed for glucose, lactate, and urea (14). Perfusate O_2 content was determined with Lex-O_2-Con (Lexington Instruments Corp, Waltham, MA). The O_2 content of the inflow cannula perfusate remained constant throughout the experimental period as determined by periodic sampling of the perfusate from the inflow cannula. Perfusate O_2 consumption was determined from the flow rate and difference in O_2 content of the inflow and outflow cannula perfusates.

Ad was determined fluorometrically as total drug equivalents in plasma and bile and as Ad after separation by thin-layer chromatography. Total fluorescence of samples was determined after extrac-

tion of 0.5-ml samples of perfusate plasma with 2 ml of ice-cold chloroform:isopropanol (1:1). Total fluorescence of each bile sample was determined after extraction with 10 ml of chloroform:methanol (2:1). Ad was estimated by the methods of Bachur et al (15). The 0.5-ml samples were extracted into chloroform:methanol (2:1), filtered with glass wool, and evaporated to dryness under nitrogen. The residue was redissolved in chloroform:methanol (2:1) and then subjected to ascending single-development chromatography on Silica Gel G thin-layer plates 250 μ (Analtech) in a chloroform:methanol:acetic acid:water (80:20:14:6) solvent system. The fluorescent areas were scraped and eluted with chloroform:methanol (2:1). Fluorescence was determined on an Aminco-Bowman spectrophotofluorometer as Ad equivalents. Activation and emission wavelengths were 485 and 585 nm, respectively. An Ad standard was chromatographed on each thin-layer plate. Recovery of Ad from samples was 80%–90%.

Data Analysis

Estimates of the maximal rates of metabolite change were determined by regression analysis (14). The significance of differences between mean values was determined by Student's t test or analysis of variance. A P value < 0.05 was considered statistically significant. Pharmacokinetic analysis was performed with the aid of a TI Programmable 59 Calculator by linear regression models or by a nonlinear least-squares regression analysis program described by Muir (16).

RESULTS

Effect of Hyperthermia and Ad on Substrate-Stimulated Biosynthesis

Livers were perfused for 1 hour at 37°C and 42°C with an initial perfusate level of 10 μg/ml of Ad. Gluconeogenesis, ureogenesis, and O_2 consumption were measured as indicators of hepatic tolerance. Lactate consumption increased and gluconeogenesis decreased in the presence of Ad at 37°C in comparison to control livers without exposure to Ad (table 1). At 42°C gluconeogenesis was significantly inhibited in the presence of Ad. Overall, the combination of hyperthermia and Ad was minimally detrimental to these hepatic functions. However, bile flow and excretion of Ad ceased at 42°C.

Perfusate samples were analyzed for Ad at intervals after the drug was mixed in the perfusion circuit. The first sample taken after mixing was the zero time for plasma decay analysis. At 37°C the initial Ad level was 2.42 ± 0.42 μg/ml, and this decreased to 0.46 ± 0.24 μg/ml within 60 minutes. At 42°C the initial Ad level was 2.32 ± 0.29 μg/ml, and this decreased to 0.53 ± 0.14 μg/ml within 60 minutes. Samples were measured at 0, 10, 30, and 60 minutes. The data best fit a single exponential curve on linear regression analysis. The correlation coefficients were 0.9. The half-life was 27 minutes at 37°C and 38 minutes at 42°C. The slopes of the decay curves were not significantly different. The hepatic tissue concentration of Ad was 21.38 ± 2.91 μg/g after 1 hour of perfusion at 37°C and 0 μg/g at 42°C.

Effect of Hyperthermia on Disposition of Ad

The 60-minute perfusions were done primarily to study the effects of Ad and temperature on hepatic biosynthetic functions. Ad levels were measured to ensure that each liver was exposed to comparable plasma drug levels. Experiments of longer duration were required to detect any differences in the disposition of Ad at elevated temperatures. In the short experiments, no substrates were added to the perfusate. For longer perfusions, glucose and amino acids were added for maintenance of hepatic functions. Since bile excretion is a primary determinant of Ad disposition and since bile flow ceased at 42°C, a temperature of 41°C was selected to allow study of bile excretion at an elevated temperature. Ad was measured by total fluorescence, which is the total Ad equivalents present including metabolites, and as Ad after separation by thin-layer chromatography. The differences between these two measurements gave an estimate of the amount of Ad converted to metabolites.

After administration of Ad into the reservoir, the perfusate plasma levels declined as shown in figure 1. The time course of the concentration of drug in the reservoir (C) may be described by the biexponential equation

$$C = Ae^{-\alpha t} + Be^{-\beta t}$$

where A and B are the zero-time concentration intercepts associated with the rapid (α) and slow (β) rate constant, respectively. The parameters A, α, B, and β from this two-compartment intravenous pharmacokinetic model were calculated by nonlinear least-squares regression analysis (16). The results are shown in table 2. The half-lives of Ad measured as total fluorescence and as parent drug were prolonged at the higher temperature, 41°C. The area under the plasma concentration-time curve (AUC) was determined on the same program by the linear trapezoidal rule and found to be unchanged for parent drug but increased for total Ad equivalents at

TABLE 1.—Effect of hyperthermia and Ad on hepatic biosynthetic functions

Parameter	μmols/g liver/min ($\overline{X}_{max}$ ± SE)*			
	Control (37°C)	Ad (37°C)	Control (42°C)	Ad (42°C)
Lactate†	−3.21 ± 0.18	−9.16 ± 3.01	−4.65 ± 0.37	−7.85 ± 1.67
Glucose‡	1.36 ± 0.07	0.52 ± 0.09	1.29 ± 0.13	−0.12 ± 0.14
Urea†	1.24 ± 0.10	1.64 ± 0.12	1.01 ± 0.10	1.35 ± 0.25
Oxygen†	4.85 ± 0.22	7.48 ± 0.72	5.79 ± 0.54	7.35 ± 1.20

*Livers from fasted rats were perfused in the presence of 10 mM lactate, 10 mM NH_4Cl, and 2.7 mM ornithine with or without Ad for 1 hr. Perfusate samples were taken every 10 mins for the 60-min perfusion. For each parameter, $\overline{X}_{max}$ refers to maximal rate of change that occurred in 1 of the 10-min periods during 60-min perfusion. Negative values for lactate and glucose indicate consumption. Significance was determined by 2-way analysis of variance.

†Significance level of < 0.05 between corresponding values for control and Ad treatment.

‡Significance level of < 0.05 between corresponding values for control and Ad treatment and between values at the 2 temperatures.

the elevated temperature, 41°C (table 2). The AUC values of 2010 μg/ml·min at 37°C and 4477 μg/ml·min at 41°C indicate marked differences in hepatic clearance of Ad between the two temperatures.

Biliary excretion of Ad was also found to be significantly decreased at 41°C in comparison to that at 37°C (table 3). This includes measurements of total Ad equivalents and parent drug. Hepatic tissue levels of Ad were 57.5 ± 14.0 μg/g at 37°C and 23.0 ± 10.7 μg/g at 41°C ($P < 0.05$). Tissue levels of total Ad equivalents were 288.7 ± 21.2 μg/g at 37°C and 322.7 ± 10.6 μg/g at 41°C ($P < 0.05$).

Bile flow at the two temperatures was not significantly altered until the third 60-minute period of the experiments (table 4).

DISCUSSION

The data demonstrate that Ad and hyperthermia were minimally detrimental to hepatic biosynthetic functions (table 1). Previous results have shown that hepatic functional integrity was maintained from 37°C to 42°C as assessed by gluconeogenesis, urea formation, and oxygen consumption (18). The increase in lactate consumption and decrease in gluconeogenesis at 37°C and 42°C are consistent with the competitive utilization of reducing equivalents for drug metabolism through the malate shuttle as noted by others (19). Gluconeogenesis from lactate and mixed-function oxidation both require oxygen and both share glucose-6-phosphate and malate, either as intermediates in the gluconeogenic sequence or as substrates for NADPH generation.

The alterations in the disposition of Ad produced by hyperthermia in the isolated perfused rat liver are partly in conflict with the results reported for rabbits subjected to whole-body hyperthermia with Ad administration (20). In the rabbits, whole-body hyperthermia was maintained for 1 hour only and the temperatures up to 42.3°C were measured with rectal probes. In this study, no differences were found in the perfusate plasma decay curves of Ad

TABLE 2.—Pharmacokinetic parameters describing distribution of Ad and total Ad equivalents in perfused rat liver at 37°C and 41°C*

	Total fluorescence		Ad	
	37°C	41°C	37°C	41°C
A (μg/ml)	4.61 ± 0.38	4.73 ± 0.27	1.20 ± 0.51	1.45 ± 0.29
α (min^{-1})	0.0429 ± 0.00081	0.0617 ± 0.00894	0.0711 ± 0.7151	0.0430 ± 0.01967
t½, α (min)	16.15 ± (8.4)	11.23 ± (3.58)	9.75 ± (8.7)	16.12 ± (6.2)
B (μg/ml)	1.92 ± 0.45	2.10 ± 0.25	2.10 ± 0.53	0.65 ± 0.35
β (min^{-1})	0.00101 ± 0.00156	0.00048 ± 0.00088	0.0041 ± 0.00212	0.0014 ± 0.00355
t½, β (min)	686 ± (444)	1444 ± (787)†	169 ± (45)	495 ± (235)†
AUC (μg/ml·min)	2010 ± (1270)	4477 ± (2713)†	530 ± (235)	502 ± (240)

*Results were calculated from mean plasma levels. SD values that were determined from analysis of all data sets are listed in parentheses; they are not calculated from the program. Only model macroconstants are listed. Estimation of model macroconstants (A, α, B, β) and their subsequent manipulation to obtain model microconstants (k_{21}, k_{12}, k_{10}) are valid and result in same values as would be obtained by estimating microconstants directly (17). t½ = half-life.

†$P < 0.05$ by t test.

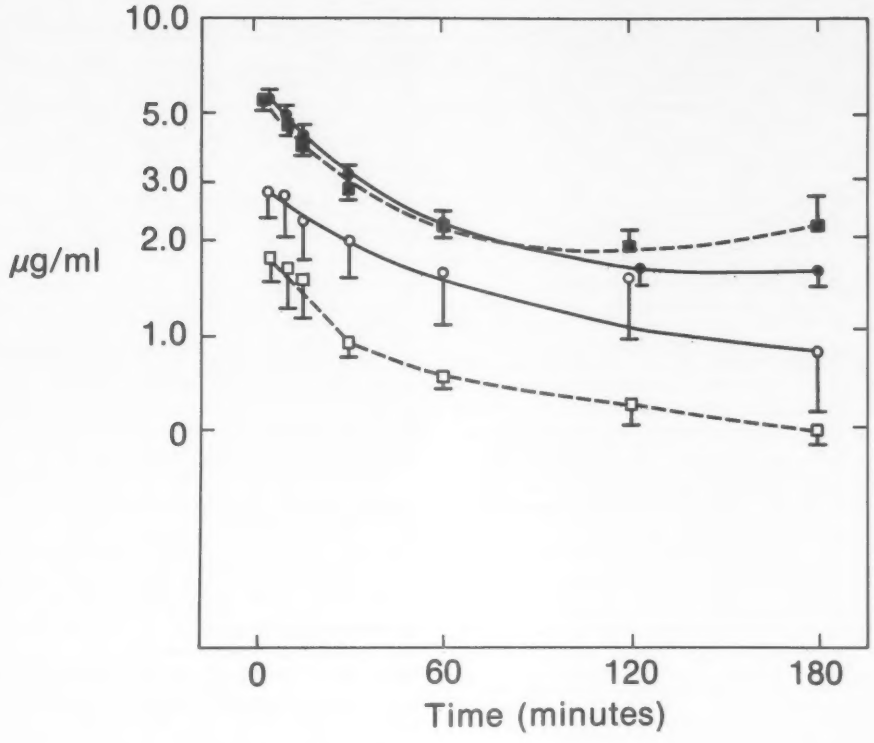

FIGURE 1.—Perfusate plasma concentrations of Ad with an initial calculated level of 15 μg/ml. Samples were obtained at 5, 10, 15, 30, 60, 120, and 180 mins after addition of drug to perfusate reservoir. Data represent mean values of 4–6 perfusions; vertical bars represent SE. ●—● = total fluorescence at 37°C, ■---■ = total fluorescence at 41°C, ○—○ = Ad at 37°C, and □---□ = Ad at 41°C.

at 37°C and 42°C; liver temperature was monitored directly with a needle probe during the 1-hour liver perfusions. One hour may not be long enough to observe significant alterations because of the long plasma half-lives of Ad.

The two-compartment open pharmacokinetic model described the plasma decay of Ad in the whole-animal experiments (13) and in these liver perfusion studies. The beta half-life of the elimination phase of the plasma concentration curve was unchanged in the rabbit by whole-body hyperthermia (13). When the perfusion time was increased to 3 hours, there were significant alterations in the beta half-lives of the elimination phase of Ad and total Ad equivalents when the temperature of the liver was increased from 37°C to 41°C (table 2). The AUC for Ad was unchanged at 41°C from that at 37°C but increased for total Ad equivalents (table 2).

The biliary excretion of Ad plus metabolites was reduced in hyperthermic rabbits to about 50% of control values (13). The biliary excretion of Ad has been studied in bile duct-cannulated rats (20–25). The percent dose of Ad excreted in the bile within 24 hours was 8%–10% (20) and 20% (21). In 3 hours after Ad administration, 25%–27% (23,24) and 4.8% (21) of the dose was found in the bile. The amount of total Ad equivalents excreted in the bile of perfused livers was 7.4% of the administered dose at 37°C (table 3). There was a significant decrease in the biliary excretion of Ad and total equivalents at 41°C.

TABLE 3.—Effect of hyperthermia on biliary excretion of Ad

	% dose excreted in 3 hrs ($\bar{X}$ ± SD)	
Temperature	Total fluorescence	Ad
37°C	7.39 ± 1.35	2.07 ± 0.37
41°C	2.56 ± 2.08*	1.31 ± 0.84*

*$P < 0.05$ by t test, n = 6.

TABLE 4.—Bile flow*

Time (mins)	µl/g liver ± SD			
	37°C		41°C	
	Control (n = 4)	Ad (n = 6)	Control (n = 4)	Ad (n = 6)
0 – 60	26.18 ± 8.29	54.52 ± 19.70	50.18 ± 10.13	43.54 ± 17.41
60 – 120	22.40 ± 6.34	44.36 ± 28.04	26.74 ± 8.52	20.51 ± 19.04
120 – 180	16.92 ± 4.85	29.45 ± 21.75	2.42 ± 0.90	2.38 ± 4.40

*Overall effect of time, ie, a decrease in bile flow, is significant at the 0.05 level. There is a significant (< 0.05) overall time-temperature interaction but no significant effect from Ad. Significance determined by 3-way analysis of variance.

The mechanism for the inhibition of biliary excretion of Ad is unknown. Cholestasis induced by heat could be a cause. When bile flow in rats was inhibited by administration of sodium taurolithocholate, the biliary excretion of total Ad equivalents decreased and the plasma concentrations increased in proportion to the degree of bile flow reduction (24). Although the biliary excretion of Ad and total Ad equivalents was decreased in this study (table 3), the plasma levels were not increased concomitantly. Moreover, the bile flow did not decrease until the third hour of perfusion (table 4). Decrease in bile flow with increasing length of rat liver perfusion is consistent with previous observations by other investigators (25–27). Brauer et al (28) demonstrated that bile production was temperature-dependent in the perfused rat liver; it was maximal between 38°C and 40°C. Both bile flow and sulfobromophthalein transport maximum were increased with a perfusate temperature of 40°C in comparison to values noted at 37°C, but the experiments were performed with the choleretic stimulus of a constant infusion of sodium taurocholate (26). Roberts et al (29) noted that bile flow in rats increased with temperature, but 39°C was the highest temperature studied.

Although bile secretory function appears to be a determinant of Ad disposition at normothermia, other mechanisms are operative at hyperthermic temperatures. The cause of decreased biliary excretion of Ad by hyperthermia could be the result of injury to the cell membrane. At 42°C there was no uptake of Ad into liver tissue. At 41°C the total amount of Ad equivalents in liver tissue was increased over that at 37°C, and the amount of intact Ad in the liver was less at 41°C than at 37°C, suggesting increased metabolism of Ad at 41°C with concomitant decrease in transport from the liver. When bile secretory function was impaired with sodium taurolithocholate, the tissue levels of Ad equivalents increased with increasing cholestasis (24). Thus, transport of Ad from the hepatocyte may be the site of hyperthermic injury. Plasma membrane proteins of several cell types undergo major structural alterations in the physiologic-hyperthermic temperature range [see review by Wallach (30)]. Heat was shown to modify membrane permeability to Ad (2,3).

There are possible clinical consequences from these changes in Ad disposition. Enhanced toxic reactions might be sequelae if Ad was administered in combination with whole-body hyperthermia, since hepatic elimination is a primary determinant of the disposition of Ad. This could perhaps be corrected by reducing the dose of Ad or administering the therapies in a sequential manner rather than concomitantly. There should be no effects on Ad disposition occurring during local hyperthermia treatments unless these treatments are directed to the liver.

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Development and Application of a Radioimmunoassay for Peplomycin[1,2]

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SUMMARY

A rapid sensitive radioimmunoassay for peplomycin (PM) has been developed using an antiserum produced in a sheep in response to a bovine serum albumin-drug conjugate. Radioiodinated PM was prepared using Iodogen. The assay was reproducible with a limit of detection in plasma of 200 ng/liter. The antiserum does not cross-react with bleomycin or with a number of unrelated drugs. Pharmacokinetic studies using the assay were carried out in three patients who received a short iv infusion of 5 mg/m² of PM. The drug disappeared from the plasma with an elimination half-life of approximately 3 hours.

[Cancer Treat Rep 66:1365–1370, 1982]

Peplomycin (PM) is an analog of bleomycin and can be distinguished from bleomycin by the substitution of a 3-[(*S*)-1′-phenylethylamino] propylamino group in the terminal amine position (1). Preclinical studies have indicated that the drug, while retaining the antibiotic and antitumor properties of bleomycin, exhibited less pulmonary toxicity than bleomycin (2,3). Despite decreased lung toxicity, PM was more lethal, with a higher mortality than bleomycin in studies on mice bearing Lewis lung carcinoma and B16 melanoma (4). In the same studies, a peculiar central nervous system toxicity was also noticed with PM.

Radioimmunoassay (RIA) techniques for measuring bleomycin (5–7) and talisomycin (8) in biologic samples have been described and are useful for defining the pharmacokinetic properties of the drug (9–11). We have found that prolonged elevated blood levels of bleomycin in a small number of patients were associated with drug toxicity (unpublished results). It is important that pharmacokinetic studies on PM be carried out during phase II and III trials on the drug, and an RIA suitable for that purpose has been developed and applied to the measurement of plasma drug concentrations in a small number of patients.

MATERIALS AND METHODS

Materials.—PM sulphate (Lot 201) was donated by Lundbeck Ltd, Luton, United Kingdom (UK). Bovine serum albumin (BSA) fraction V, bovine gamma globulin (BGG), ethyl (dimethyl amino propyl)-γ-carbodiimide (EDC), Norit A charcoal, and gelatin were purchased from Sigma Chemicals Co, Ltd, Dorset, UK, Sephadex G10 and Dextran T70 from Pharmacia, Uppsala, Sweden, Iodogen (1,3,4,6-tetra-chloro, 3α-6α-diphenylglycol uril) from Pierce and Warriner, Chester, UK, and $Na^{125}I$ (IMS 30) from Amersham International Ltd, Amersham, UK. Other chemicals were of reagent grade and obtained from BDH Chemicals Ltd, UK. Half lop rabbits were obtained from Ranch Rabbits Ltd, Crawley, UK.

Preparation of immunogen.—PM (45 mg) and BSA (50 mg) were dissolved in 2.5 ml of distilled water,

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EDC (35 mg) was added, and the solution was stirred overnight. In a similar reaction, PM (22.5 mg), BGG (25 mg), and EDC (17.5 mg) were mixed in 1.25 ml of water for 3 hours. Unconjugated PM was removed by dialysis against several changes of 500 ml of distilled water. The amount of unconjugated drug was determined by ultraviolet analysis (285 nm) of the dialysates. The molar incorporation of PM was 9 and 6 for the BSA and BGG conjugates, respectively. The immunogens were stored at 4°C at an approximate protein concentration of 5 g/liter.

Immunization procedures.—One Soay sheep and one rabbit were injected with the PM-BSA conjugate, and two rabbits were injected with the PM-BGG conjugate. The injections were prepared by emulsifying one volume of aqueous conjugate with two volumes of nonulcerative complete Freund's adjuvant (12). The priming injection contained 5 mg of conjugate for the sheep and 2.5 mg of conjugate for the rabbits. The animals were injected in 4–6 intramuscular sites on the legs and back. Booster immunizations were carried out at intervals using half the quantity of immunogen in incomplete adjuvant. At intervals following the injections, blood was collected from the jugular vein of the sheep and the lateral ear veins of the rabbits. The serum was separated and stored at 4°C after the addition of sodium azide at a final concentration of 0.1% (wt/vol).

Preparation of radioiodinated PM.—Iodogen (20 μg) was deposited from dichloromethane (20 μl) onto the bottom of a polypropylene tube (Luckhams Ltd, LP3P). Five micrograms of PM sulphate in 10 μl of 0.2 M borate buffer (pH 8.2) and 0.5 millicurie of $Na^{125}I$ were added to the tube, and the tube was capped and gently vibrated for 2 minutes. A 200-μl volume of 0.1 M phosphate buffer (pH 7.4) was used to transfer the contents of the tube to a Sephadex G10 column (1 × 10 cm), and radioiodinated drug was eluted in 1-ml fractions with assay buffer (*see* assay procedure). The fraction containing the most radioactivity, eluted with 5–6 ml of buffer, was retained and stored at 4°C for use in the RIA.

Assay procedure.—The assay buffer used throughout the procedure (table 1) was 0.05 M phosphate (pH 7.4) containing 1 g/liter of gelatin and 6 g/liter of NaCl. All dilutions and additions were performed using Compupet (Warner Diagnostics Ltd, Eastleigh, UK) or Oxford dispensers. ^{125}I-PM was diluted immediately before use so that approximately 12,000 cpm was added to each tube. The antiserum was diluted so that 50% of the immunoreactive label (maximum bound in the presence of excess antibody) was bound in the zero standard tube. PM sulphate (100 mg/liter) in 0.05 M phosphate buffer (pH 7.4) was stored for up to 2 months at 4°C and was diluted immediately before use as the standard in the assay. Samples were assayed in duplicate at three or four appropriate dilutions. The reagents were added in the order indicated in table 1 to plastic tubes (Luckhams Ltd, LP3), mixed, and incubated at 4°C for 1 hour. Dextran-coated charcoal, prepared as described earlier (13), was added, and after 10 minutes, the tubes were centrifuged, the supernatant was aspirated, and the charcoal pellet was counted.

RESULTS

Antiserum and Label Preparation

PM antisera were obtained following the priming injection from all the immunized animals. Subsequent booster injections increased the antiserum

TABLE 1.—Protocol for RIA of PM

Reagent	Volume of reagents added (μl)				
	Total counts tube	Nonspecific binding tube	Maximum binding tube	Zero tube/ antiserum dilution curve	Standard or sample tube
Assay buffer	—	500	—	400	300
Standard/sample	—	—	—	—	100
Antiserum dilution	—	—	500	100	100
^{125}I-PM	100	100	100	100	100
Mix and incubate at 4°C for 1 hr					
Dextran-coated charcoal	—	100	100	100	100
Mix and incubate at 4°C for 10 mins					
Centrifuge, aspirate supernatant, and count charcoal pellet					

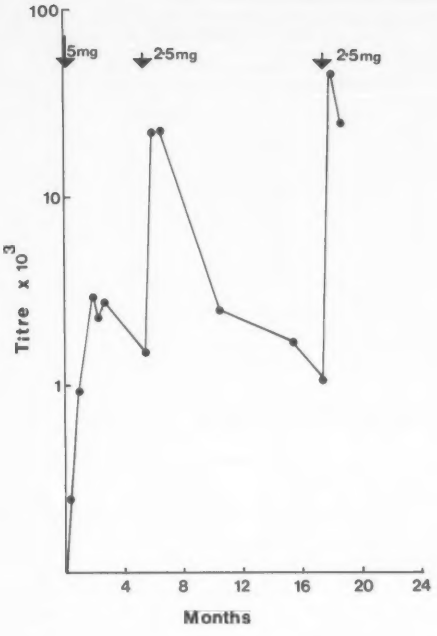

FIGURE 1.—Production of antibodies in response to PM-BSA in sheep G/S/684. Titer was that dilution of antiserum which bound 50% of immunoreactive ^{125}I-PM.

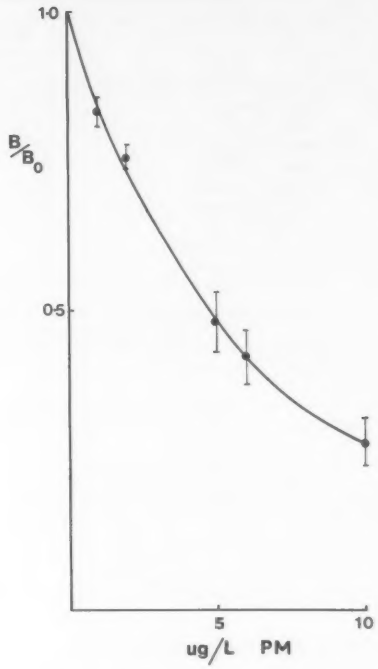

FIGURE 2.—Mean standard curve for PM (n = 10) ± SD. B/$_{B_0}$ is the ratio of antibody-bound label at standard concentration to antibody-bound label at zero standard concentration.

titers considerably. Preimmunization sera did not bind the iodinated drug. Figure 1 shows the antibody response to immunogen in the sheep G/S/684 immunized with PM-BSA. A pool of antisera collected following the first booster injection (22 weeks) was subsequently used to develop and validate the RIA and could be used at an initial dilution of approximately 1:20,000, depending on the specific activity of the batches of tracer used.

Radioimmunoassay

The displacement of ^{125}I-PM from antibody binding sites by PM is shown in figure 2 and is the mean curve of ten consecutive standard curves. Table 2 shows the within-assay and between-assay variation of the standard curve. Standard curves obtained at 4°C with incubations for 1 and 3 hours, and at room temperature with incubations for 1 hour and 24 hours were identical; for practical reasons it was decided to use an incubation time of 1 hour at 4°C.

Standard curves were constructed with 0, 10, 20, and 50 μl of normal pooled drug-free serum and 0, 5, and 10 μl of normal human pooled urine added. The maximum volume of sample that would be added to the assay is represented by 50 μl (ie, dilution of 1–2) although most samples would be assayed at considerably higher dilutions. There was no effect of either serum or urine on the shape of the standard curve or the percent of binding.

The theoretic limit of detection of the RIA was calculated from the slope of the curve and the standard deviation of the zero point (14) to be 100 ng/liter. The avidity of the antiserum was determined to be 8×10^9 L/M using a Scatchard plot (15) and 7.0×10^9 L/M using a method described by Muller (16). Bleomycin did not inhibit the binding of ^{125}I-PM to antibody at bleomycin concentrations as high as 100 mg/liter, which represents a cross-reactivity of much less than 0.005% compared with PM. Methotrexate, vincristine, etoposide, cytarabine, 5-FU, and doxorubicin, as well as a number of noncytotoxic drugs, did not cross-react with the antibody at concentrations of 100 mg/liter.

TABLE 2.—Within- and between-batch variation of the PM standard curve (n = 10)*

	Within-batch variation		Between-batch variation	
	Mean ± SD	CV%	Mean ± SD	CV%
Total (T) cpm	11629 ± 64	0.6		
NSB (% T)	0.65 ± 0.47	7.2		
MB (% NSB)	91.1 ± 0.53	0.6	90.9 ± 1.8	2.0
Zero (% NSB)	61.9 ± 1.0	1.6	61.5 ± 4.2	6.8
1 ng/ml (% zero)	79.2 ± 3.75	4.7	83.2 ± 2.7	3.3
2 ng/ml (% zero)	72.9 ± 1.37	1.9	75.8 ± 2.3	3.1
5 ng/ml (% zero)	45.0 ± 3.15	7.0	48.1 ± 5.3	11.0
6 ng/ml (% zero)	38.2 ± 2.39	6.0	42.3 ± 4.6	10.7
10 ng/ml (% zero)	24.5 ± 2.09	8.5	28.2 ± 3.9	13.7

*NSB (nonspecific binding) was calculated as $\frac{\text{total cpm} - \text{NSB cpm}}{\text{total cpm}} \times 100$. MB (maximum binding using excess antibody) and zero standard binding were calculated as a % of the cpm in the NSB tubes. The standard curve was calculated as a % of zero standard binding. CV = coefficient of variation.

TABLE 3.—Recovery of drug from a pool of normal drug-free human serum spiked with PM

Concentration of PM in spiked normal human serum	% recovered ± SD	n	CV%
1 mg/liter	117.4 ± 14.4	10	12.2
100 μg/liter	99.0 ± 11.6	7	11.7
10 μg/liter	97.7 ± 11.5	9	11.8

RIA of PM in Plasma Samples

The recovery of PM added to a normal drug-free plasma pool is shown in table 3. PM concentrations were measured (and the assay repeated over a period of 2 months) in plasma samples obtained from two patients given 5 mg/m² of PM iv over 10 minutes (table 4). The amount of PM bound to plasma proteins was determined in two plasma pools using equilibrium dialysis experiments. Small circular perspex cells (volume, 2 ml) divided by a single layer of Visking tubing were used; 1 ml of plasma was placed on one side of the membrane and 1 ml of 0.1 M phosphate buffer (pH 7.4) was placed on the other side. The cells were closed and rotated overnight at room temperature. The concentration of PM on each side of the dialysis membrane was measured using the RIA. Assuming that equilibrium had been reached and that there had been no binding of drug to the membrane itself, the amount of drug measured on the buffer side of the membrane (the dialysate) was equal to 50% of the free drug, ie, nonprotein-bound drug. The results are shown in table 5.

The disappearance of PM in plasma over 20 hours following the administration of drug (5 mg/m² by 10-minute infusion) was measured in three patients[6] (fig 3). The results are expressed as immunoreactive PM, since the specificity of the antiserum towards metabolites of PM is not known. Results were analyzed according to a two-compartment model following an iv bolus injection. The equation $C_t = A_e^{-\alpha t} + B_e^{-\beta t}$ was calculated for each patient by a nonlinear regression analysis of the data using a BASIC program for a Hewlett-Packard 85A microcomputer. Pharmacokinetic parameters were calculated from the computed values of the constants in the equation and are shown in table 6.

[6]Plasma samples were supplied by Dr. P. G. Sorenson, Finsen Institute, Copenhagen, Denmark.

DISCUSSION

A sensitive RIA for PM, which is suitable for pharmacokinetic studies on the drug, has been developed. The antiserum used in the assay was produced in response to a protein conjugate prepared in a manner similar to that described for bleomycin. Specificity studies on bleomycin antisera have shown that alteration of the chemical structure in the nucleus of the molecule, or absence of the terminal side chain, eliminated antibody binding. Chemical modifications in the terminal side chain were recognized by the antisera to various extents and the metabolite desamido-bleomycin cross-reacted significantly (17).

Although specificity studies other than with bleomycin have not yet been carried out, it can be pre-

TABLE 4.—Between-batch variation of the measurement of PM in plasma samples

	Time (mins)	Concentration of PM (μg/liter) ± SD	n	CV%
Patient A	30	422 ± 28.4	5	6.7
	40	663 ± 54.7	5	8.7
	60	363 ± 49.9	6	13.7
Patient B	5	1495		
	20	729		
	40	625		
	60	663		
Pool 1*		825 ± 43.5	10	5.3
Pool 2*		653 ± 32.8	10	5.0

*Samples were randomly pooled from patients A + B for between-batch assays.

TABLE 5.—Measurement of free and plasma-bound PM by equilibrium dialysis

	PM concentration in g/ml						
Pool	Undialyzed plasma pool	Dialysate (F)	Dialyzed plasma pool (B)	Amount recovered (%)	Free drug (2 × F)	Protein-bound drug (B − F)	% bound
1	851	217	365	582 (68.5)	434	148	25.4
	650	157	368	525 (80.7)	314	211	40.0
2*	607	186	363	549 (90.6)	372	177	32.2

*Protein binding in pool 2 was determined on 2 occasions.

dicted that the PM antiserum used in this assay will display characteristics similar to those of the bleomycin antisera concerning structural changes in the molecule. Thus, because the presence and number of any metabolites of PM and their effect on the binding of the antibodies is presently unknown, results are expressed as "immunoreactive PM." However, the assay is specific as far as a number of unrelated drugs are concerned and will be useful for pharmacokinetic studies in patients who receive a number of cytotoxic and other drugs.

The detection limit of the assay is more than adequate for pharmacokinetic studies and allows the assay to be carried out on low sample volumes. Increased sensitivity where 20 pg of drug can be detected can be achieved using ^{125}I-PM prepared with 1 millicurie of ^{125}I, but the between- and within-batch variations obtained were unsatisfactory. Tracers prepared with 0.5 millicurie of ^{125}I, at the expense of a reduction in sensitivity, resulted in acceptable reproducibility.

The recovery of added drug from serum was complete, although for the 1-mg/liter concentration it was > 100%. The between-batch variation of the assay is good, and it appears that samples can be stored for at least 2 months at 4°C without deterioration.

Preliminary pharmacokinetic studies have indicated that following a short infusion PM disappears from plasma in a biphasic fashion, with an elimination half-life of approximately 3 hours. This figure compared with the half-lives quoted for bleomycin following various routes of administration (18,19).

The dialysis experiment showed that 25%–40% of PM was protein-bound. This is in contrast to results reported for bleomycin (9) where < 1.0% of the drug was protein-bound. The bleomycin experiments were

TABLE 6.—Computed PM pharmacokinetic parameters

Parameter	Mean value ± SEM (n = 3)
A (μg/ml)	0.056 ± 0.19
B (μg/ml)	0.029 ± 0.03
α (hr^{-1})	0.98 ± 0.18
β (hr^{-1})	0.22 ± 0.01
$t_{1/2}\alpha$ (hr)	0.77 ± 0.17
$t_{1/2}\beta$ (hr)	3.10 ± 0.17
Kel* (hr)	0.46 ± 0.11
V_d area† (liters)	12.1 ± 1.07
Mean plasma clearance (ml/min)	45.0 ± 3.48

*Kel = first-order rate constant for elimination of drug from central compartment.

†V_d = apparent volume of distribution calculated from the area under the curve.

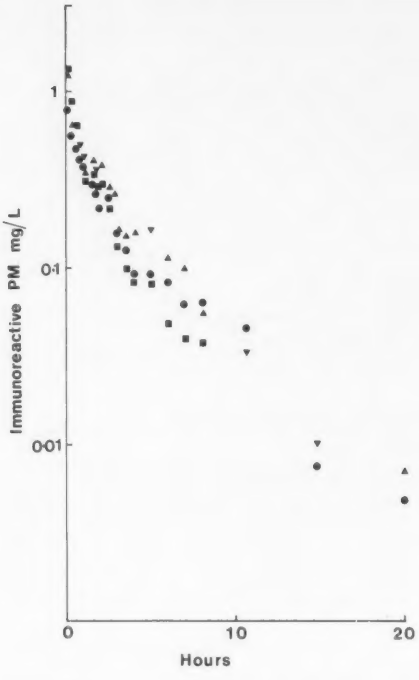

FIGURE 3.—Disappearance of PM from the plasma of 3 patients (● ■ ▼) given 5 mg/m² iv over 10 mins. Blood samples were taken at intervals from the end of the infusion. *See* table 5 for pharmacokinetic data.

carried out after incubation of drug with normal human serum "in vitro," whereas the present study on PM used plasma pools from patients after PM administration. The difference in protein binding of bleomycin and PM may be important in interpreting pharmacokinetic data, and further studies are required to confirm our findings. The availability of an RIA and a recently reported enzyme immunoassay (20) for PM will allow extensive pharmacokinetic studies on the drug in various clinical situations, and these studies may be important in relating toxicity to individual differences in drug elimination.

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Prevention of Doxorubicin-Induced Skin Ulcers in the Rat and Pig With Dimethyl Sulfoxide (DMSO)[1,2,3]

Manubhai H. Desai* and Daniel Teres[4,5]

SUMMARY

Ten potential agents were tested in the Sprague-Dawley rat, using the method of Rudolph et al, which produces skin ulcers with 0.4 mg of doxorubicin intradermally. One antidote, dimethyl sulfoxide (100%, 0.6 ml for 7 days), prevented ulcer formation not only at the local site but also at the doxorubicin control injection site. Similar ulcers were produced in the Yorkshire pig with 2.0 mg of intradermal doxorubicin injection. These ulcers were prevented at both local and distant control sites with dimethyl sulfoxide given for 7 days.

[Cancer Treat Rep 66:1371–1374, 1982]

Doxorubicin, a potent antineoplastic drug, is effective in adult and childhood solid tumors and hematologic cancer. Apart from minor toxic effects such as mucositis, nausea, vomiting, and alopecia, the three major clinical toxic effects are myelosuppression, cumulative dose-related cardiomyopathy, and severe, prolonged skin and subcutaneous ulcers resulting from paravenous drug extravasation.

Cutaneous and subcutaneous ulcerations following paravenous extravasation can be caused by several antineoplastic agents. The possible unique aspects of anthracycline-induced ulcers are their prolonged course with slow healing and their propensity to invade deep structures such as tendons and joints without respect for fascial planes (1).

In 1979, Rudolph et al (2) were able to produce ulcers of predictable size by intradermal rather than sc injection of doxorubicin in laboratory animals. They found that the diameter of the ulcer produced by intradermal doxorubicin injection was related to both the concentration of doxorubicin and the volume of injection. Generally, 0.2 ml of doxorubicin at a concentration of 2 mg/ml produced a 1-cm-diameter lesion that took 7 or 8 weeks to heal.

Apart from measures to prevent paravenous extravasation, many methods of preventing these ulcers have been tried clinically, but the efficacy is uncertain. Suggested methods include subcutaneous injection of sodium bicarbonate or corticosteroids (3) and/or ice pack for 24 hours (4). Excision and grafting have also been recommended. The following study was undertaken to test a large number of potential counteracting agents.

MATERIALS AND METHODS

Sprague-Dawley male rats weighing between 200 and 300 g, with their backs shaved, were used. Doxorubicin and/or antidotes were injected intradermally in the shaved areas. Each rat was kept separately in a cage and was given water and laboratory food ad libitum. The following series of experiments were performed. Doxorubicin (2 mg/ml) dissolved in physiologic saline was used in all experiments.

The first experiment was performed to confirm the results of Rudolph et al (2) and to standardize doxorubicin injections. Three rats in each group were injected intradermally using a 1-ml syringe and a

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25-gauge needle. Physiologic saline and normal saline with 0.9% benzyl alcohol were both used as control injections. The quantity of doxorubicin (2 mg/ml) injected at each site was 0.1, 0.15, 0.2, 0.25, or 0.3 ml. In these rats, weekly measurements of the widest perpendicular diameter of the ulcers were taken with a millimeter ruler. The rats were also photographed weekly and were killed at 8 weeks.

The second experiment screened ten potential agents counteracting doxorubicin skin necrosis in three rats each. The volume of counteracting agent injected at each site was 0.6, 0.3, or 0.15 ml. Physiologic saline and 0.2 ml of doxorubicin were used at control sites. The agents tested were sodium bicarbonate, normal saline, 0.5% benzoic acid, *N*-acetylcysteine, alpha-tocopherol, glutathione, coenzyme Q_{10}, carnitine, methylprednisolone, and dimethyl sulfoxide (DMSO; Sigma Chemical Co, St. Louis, MO). The rats were observed for 4 weeks.

In the third experiment, counteracting agents were screened in three rats immediately after they had each received intradermal injections of doxorubicin at three sites. The volumes of counteracting agents used were 0.6, 0.3, and 0.15 ml. Doxorubicin and saline (both at 0.2 ml) acted as controls in each rat at the fourth and fifth sites. The animals were observed for 4 weeks, with weekly photographs.

In the fourth experiment, DMSO was further tested. Groups of four animals each were injected intradermally with 0.2 ml of doxorubicin. Three animals in each group received DMSO topically (0.6, 0.3, or 0.15 ml) for 7 days. The fourth animal acted as a concurrent control. The animals were observed for 4 weeks.

In the fifth experiment, two groups of three rats each received intradermal doxorubicin (0.2 ml) at two sites each. Each animal in one group was painted with 0.6 ml of DMSO for 7 days at one site only. The second group was painted with 0.6 ml of DMSO at one site for 1 day only. The fourth animal in each group received intradermal doxorubicin (0.2 ml) only.

Because pig skin resembles human skin, the production of doxorubicin-induced skin ulcers was adapted and modified in three 6-week-old Yorkshire pigs. Each pig was injected intradermally with 0.25, 0.5, 0.75, and 1.0 ml of doxorubicin (2 mg/ml) at four different sites. Nine other Yorkshire pigs were injected with 1.0 ml of doxorubicin at two different sites. In three of these pigs, 3 ml of DMSO was painted at one site for 1 day only. In six of these pigs, DMSO was painted daily for 7 days at one site only, with 3 ml of DMSO in three pigs and 1.5 ml in three. The animals were observed for 4–8 weeks, and ulcer diameters were measured and photographs were taken weekly.

RESULTS

We confirm the results of Rudolph et al (2) that 0.2 ml of doxorubicin (2 mg/ml) produces an ulcer approximately 1 × 1 cm. These ulcers develop at 1 week, remain stable for 4–5 weeks, and heal by 7 or 8 weeks. The potential counteracting agents alone did not produce skin ulcers and had no side effects in 4 weeks of observation. When the potential counteracting agents were injected immediately after 0.2 ml of doxorubicin, only DMSO prevented ulcers. In two animals it also reduced the size of the control ulcer, which healed quickly, and in one animal, the control ulcer did not develop (table 1). There was some protection from sodium bicarbonate as well as vitamin E as compared to saline in this screening experiment. The ulcer was smaller with the saline diluent than with any other agent.

In each of six rats, 0.6, 0.3, or 0.15 ml of DMSO was painted daily for 7 days on one site injected intradermally with 0.2 ml of doxorubicin. No ulcers developed in the injection sites painted with 0.6 and 0.3 ml of DMSO. Small ulcers developed in those areas painted with 0.15 ml of DMSO.

In each of six other rats, 0.2 ml of doxorubicin was injected in two separate sites. Three of these rats were painted at one site only for 7 days with 0.6 ml of DMSO. No ulcers developed at either site. Sites on the other three rats were painted on 1 day only. Two rats developed small ulcers at both sites, which healed in 4 weeks. The third rat did not develop any ulcer. Separate concurrent controls developed ulcers 1 × 1 cm.

In three control pigs, 1.0 ml of doxorubicin (2 mg/ml) produced an ulcer approximately 1 × 1 cm at 1 week (fig 1). This ulcer remained stable for 4 weeks. In the three pigs that received DMSO once only at one site, ulcers developed at both control and treated sites at 2 weeks, but the ulcers were approximately one half the size of the ulcers in untreated control animals. Six other pigs that were painted at one site only with DMSO (3 ml in three pigs and 1.5 ml in three pigs) daily for 7 days did not develop ulcers at either site.

DISCUSSION

The aglycone portion of doxorubicin contains a quinone and a hydroquinone ring. Misra and Fridovich (5) have shown that quinone-containing compounds generate active oxygen species during their redox cycle. The formation of superoxide in a liver microsomal system by anthracycline antibiotics was first shown by Handa and Sato (6). Superoxide radical ions can decompose to yield hydroxyradicals,

TABLE 1.—Ulcer diameters at end of Week 1 after injection of doxorubicin* and various doses of potential counteracting agents at different sites

Agent	Rat No.	Ulcer diameter (in cm) following injection with—				
		Saline (0.2 ml)	Doxorubicin	Doxorubicin + 0.6 ml of agent	Doxorubicin + 0.3 ml of agent	Doxorubicin + 0.15 ml of agent
Glutathione (100 mg/ml)	1	0.0	1.0	0.4	0.7	0.5
	2	0.0	1.0	0.6	0.6	0.6
	3	0.0	1.0	0.6	0.7	0.6
DMSO (100%)	1	0.0	0.3	0.0	0.0	0.0
	2	0.0	0.2	0.0	0.0	0.0
	3	0.0	0.0	0.0	0.0	0.0
L-Carnitine (100 mg/ml)	1	0.0	1.0	0.35	0.45	0.5
	2	0.0	1.0	0.4	0.4	0.5
	3	0.0	1.0	0.4	0.4	0.4
Benzoic acid (0.5%)	1	0.0	1.0	0.5	0.6	0.55
	2	0.0	1.0	0.3	0.4	0.4
	3	0.0	1.0	0.3	0.3	0.5
Alpha-tocopherol (100 units/ml)	1	0.0	1.0	0.2	0.2	0.9
	2	0.0	1.0	0.3	0.3	0.3
	3	0.0	1.0	0.2	0.3	0.2
Sodium bicarbonate (50 mEq/50 ml)	1	0.0	1.0	1.2	0.7	1.0
	2	0.0	1.0	0.2	0.3	0.3
	3	0.0	1.0	0.3	0.2	0.2
Methylprednisolone (40 mg/ml)	1	0.0	1.0	0.7	0.5	0.5
	2	0.0	1.0	0.9	1.0	0.5
	3	0.0	1.0	0.8	1.0	0.5
Coenzyme Q_{10} (5 mg/ml)	1	0.0	1.0	0.8	0.5	0.6
	2	0.0	1.0	0.8	0.5	0.5
	3	0.0	1.0	0.5	0.4	0.6
Bacteriostatic saline with benzyl alcohol (0.9%)	1	0.0	1.0	0.8	0.4	0.5
	2	0.0	1.0	0.8	0.5	0.4
	3	0.0	1.0	0.3	0.3	0.4
N-Acetylcysteine (100 mg/ml)	1	0.0	1.0	0.6	0.5	0.5
	2	0.0	1.0	0.7	0.5	0.4
	3	0.0	1.0	0.5	0.8	0.6

*0.2 ml of doxorubicin (2 mg/ml).

peroxyradicals, and hydrogen peroxide. Such oxygen-free radicals may initiate a peroxidation of endogenous lipids and cause membrane damage (7).

Alpha-tocopherol in DMSO (90%) was shown by Svingen et al (8) to reduce the size of ulcer. DMSO was used as a carrier agent for the relatively insoluble alpha-tocopherol. In their 2-day study, DMSO alone seemed to reduce the size of ulcers both on the treated and untreated flanks of separate controls. Their statistical comparison was made between the control on the opposite flank and not with separate control animals. In our study, DMSO not only prevented ulcer formation at local sites but also at distant sites. Vitamin E alone was not tried by Svingen et al. In our screening experiment with a small number of animals, there was some reduction in the size of ulcers treated with vitamin E. Inability to penetrate and to distribute along tissue planes may be the problem with other free-radical scavengers. There may also be differences in the manufacturing of DMSO or in the concentration or method of application.

DMSO, as a drug solvent that penetrates along all tissue planes, may reduce the concentration of doxorubicin. However, it is unlikely that doxorubicin was extracted out of the skin by DMSO, because DMSO worked as effectively at distal sites. DMSO is a potent hydroxyl radical scavenger (9). If doxorubicin-induced skin ulcers are related to the formation of toxic free radicals of oxygen, then DMSO may prevent ulcers by scavenging of free hydroxyl radicals. Further work is needed in animal models more closely related to human extravasation problems. Also needed is more work in agent combinations, dose- and time-related studies, and pharmacodynamics.

FIGURE 1.—Photograph of a pig showing doxorubicin-induced skin ulcers.

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Prevention and Reversal by a Retinoid of 3,4-Benzpyrene- and Cigarette Smoke Condensate-Induced Hyperplasia and Metaplasia of Rodent Respiratory Epithelia in Organ Culture[1]

Ilse Lasnitzki[2,*] and Werner Bollag[3,4]

SUMMARY

The influence of an aromatic analog of vitamin A, etretinate, on the effects of 3,4-benzpyrene and cigarette smoke condensate has been investigated in fetal mouse lung and neonatal rat tracheas grown in organ culture. In both tissues, 3,4-benzpyrene as well as cigarette smoke condensate induces a striking increase of epithelial mitosis within 12–14 days of treatment. The increase is associated with a loss of secretory activity and of ciliary function. These changes persist in the absence of benzpyrene or smoke condensate in explants transferred to control medium. Treatment with etretinate alone does not affect the normal epithelial growth rate or normal differentiation. If combined with either benzpyrene or smoke condensate, the aromatic compound inhibits the increase in cell division and prevents the loss of secretory activity or ciliary function. In explants pretreated with 3,4-benzpyrene or cigarette smoke condensate, etretinate reduces the carcinogen- or smoke condensate-induced increase in mitotic activity to normal levels and restores secretory differentiation and ciliary function. The mechanism of action involved in the anticarcinogenic activity of the retinoid is discussed.

[Cancer Treat Rep 66:1375–1380, 1982]

Vitamin A is essential for the maintenance of growth, vision, and reproduction and for the differentiation of epithelia. In vitamin A deficiency, epithelia of the respiratory tract and of male accessory sex organs undergo squamous metaplasia. (1–3). Conversely, excess vitamin A induces a mucous metaplasia of squamous keratinizing epithelium (4–7).

There is mounting evidence that, in addition to its physiologic function, vitamin A also possesses anticarcinogenic properties. This was first seen in mouse prostate glands in organ culture in which vitamin A counteracted the effects of the carcinogenic hydrocarbon 3-methylcholanthrene (8,9) and was confirmed in animal experiments. The induction of respiratory tumors in rats and hamsters and that of skin papillomas and carcinomas in mice was inhibited by treatment with vitamin A or vitamin A acid (10–12).

However, the use of vitamin A or vitamin A acid as potential anticancer agents is restricted by their toxicity at pharmacologic concentrations, and in an attempt to reduce the toxicity, a number of structural analogs (retinoids) have been synthesized (13). Among these retinoids is the aromatic retinoid etretinate, in which the cyclohexenyl ring of the ethyl ester of retinoic acid has been replaced by a substituted aromatic ring. This retinoid is highly active in prevention of and therapy for DMBA-induced skin papillomas and carcinomas in mice and possesses a particularly favorable ratio between anti-

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tumor activity and toxic effects (13–15). Furthermore, it reverses methylcholanthrene-induced hyperplasia and metaplasia of prostatic epithelium in vitro (16) and was seen to inhibit the growth of a human bronchial carcinoma implanted into nude mice (17).

The response of secretory epithelia and in particular that of bronchial epithelium to this retinoid prompted the question of whether it may inhibit or delay the early effects of cigarette smoke and its hydrocarbons on respiratory epithelia in general, and it seemed worthwhile to examine the interaction of this potent retinoid and carcinogenic compounds in greater detail and under strictly controlled experimental conditions. The present paper reports experiments in which the influence of etretinate on the effects of 3,4-benzpyrene and cigarette smoke condensate has been investigated in rodent bronchial and tracheal epithelium grown in organ culture. The results may provide valuable clues for clinical trials to assess the influence of etretinate on the progression of precancerous and cancerous conditions of the human respiratory tract.

MATERIALS AND METHODS

The tissues used for the experiments were 17–18-day fetal mouse lungs from a laboratory inbred strain of R mice and tracheas from neonatal rats obtained from a strain of hooded Lister rats. They were grown in organ culture by a modified Trowell technique (18,19). The lungs were divided into fragments measuring approximately 2 × 2 × 3 mm and the tracheas were halved. Six to eight lung fragments and four half-tracheas were explanted on strips of Millipore filters which were placed on grids of stainless steel. The grids rested in small culture chambers filled with medium up to the level of the rafts. The culture chambers were placed in Petri dishes carpeted with moist filter paper to provide a humid atmosphere. The medium used was Parker's 199 (20) supplemented with 15% newborn calf serum which had been irradiated with ultraviolet light to inactivate the retinol present in the serum. Before incubation, the explants were perfused with a mixture of 5% CO_2 and 95% oxygen for 20 minutes at a flow rate of 125 ml/min.

3,4-Benzpyrene or hydrocarbon-enriched neutral fraction of cigarette smoke condensate was added to the medium at concentrations of 5 or 30 μg/ml, respectively. The retinoid etretinate (supplied by F. Hoffmann-La Roche and Co, Ltd) was used at a concentration of 2 μg/ml.

In a first series of experiments, tracheal explants were grown for 14 days with either 3,4-benzpyrene or cigarette smoke condensate alone or combined with the retinoid; lung explants were grown for 14 days with 3,4-benzpyrene or for 12 days with cigarette smoke condensate alone or combined with the retinoid.

In a second series of experiments, both tissues were first exposed to the carcinogen or cigarette smoke condensate for 12 or 14 days and then transferred to control medium or medium containing etretinate for a further 4 days. To assess the effect of the retinoid alone, additional sets of explants were treated with etretinate for 12 or 14 days or explants kept previously in control medium were exposed to the retinoid for 4 days.

At the end of the culture period, the explants were incubated with demecolcine (2.0 μg/ml), a metaphase-arresting agent, for 5 hours and then fixed in Bouin's solution, dehydrated, embedded in paraffin, serially sectioned, and stained with hematoxylin-eosin or by the Azan substitute method.

The criteria of effect used were changes in cell differentiation and cell proliferation. The latter was quantitated and expressed as the percent of epithelial mitoses and its standard deviation.

RESULTS

Fetal Mouse Lung

Before explantation, the lungs of 17- or 18-day mouse embryos consist of primitive bronchioli lined with cuboidal epithelium and embedded in cellular connective tissue. Within 12–14 days of growth in control medium, a network of branching bronchi has developed. They are lined with tall secretory cells which show a small number of cell divisions. Addition of the retinoid alone does not affect this normal pattern of growth.

Exposure to 3,4-benzpyrene or smoke condensate increases epithelial mitosis leading to hyperplasia of the bronchial epithelium. In some areas, the hyperplastic epithelium is composed of proliferating basal cells surmounted by the original secretory epithelium, and in others, the superficial secretory cells are shed. In explants pretreated with either benzpyrene or smoke condensate and transferred to control medium, the increase in cell proliferation and hyperplasia persists or progresses in the absence of the carcinogens, and in most hyperplastic foci secretory cells are now absent.

Addition of the retinoid inhibits the induction of hyperplasia and maintains secretory activity if combined with the carcinogens; if administered afterwards, the retinoid reverses the hyperplasia and restores secretory activity.

FIGURE 1.—Prevention of benzpyrene (BP)- and cigarette smoke condensate (S)-induced mitotic stimulation of bronchial epithelium by etretinate (E). Co = control medium.

The mitotic counts support these histologic observations; they are summarized in figures 1 and 2. Treatment with 3,4-benzpyrene raises the incidence of mitosis to over four times the control value, and the cigarette smoke condensate increases it more than twofold. The retinoid alone does not affect the normal mitotic rate but it does prevent the carcinogen- and smoke condensate-induced increase during combined treatment.

In explants pretreated with 3,4-benzpyrene and transferred to control medium, the incidence of mitosis is nearly six times that of the control value and more than threefold in those previously exposed to the smoke condensate. Application of the retinoid alone for 4 days does not alter the normal incidence of mitosis but it reverses the augmentation induced by the carcinogen or smoke condensate.

Rat Trachea

In neonatal rats, the trachea is lined with one row of cuboidal, occasionally ciliated cells, separated from the surrounding ring of cartilage by a thin layer of connective tissue. After 12 or 14 days in control medium, the epithelium has become columnar due to an enlargement of the supranuclear cytoplasmic

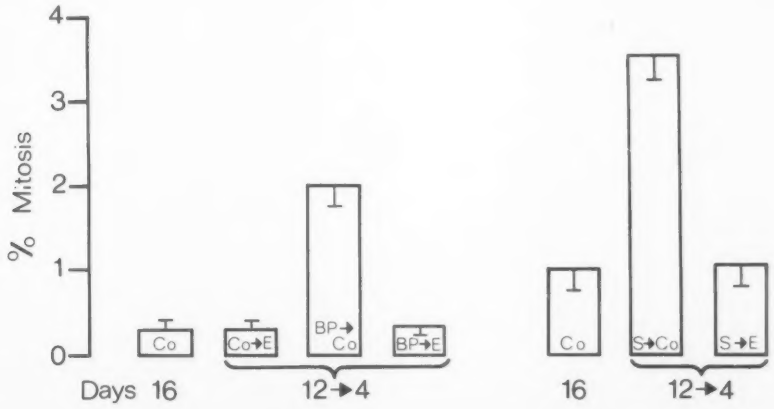

FIGURE 2.—Reversal of benzpyrene (BP)- and cigarette smoke condensate (S)-induced mitotic stimulation of bronchial epithelium by etretinate (E). Co = control medium.

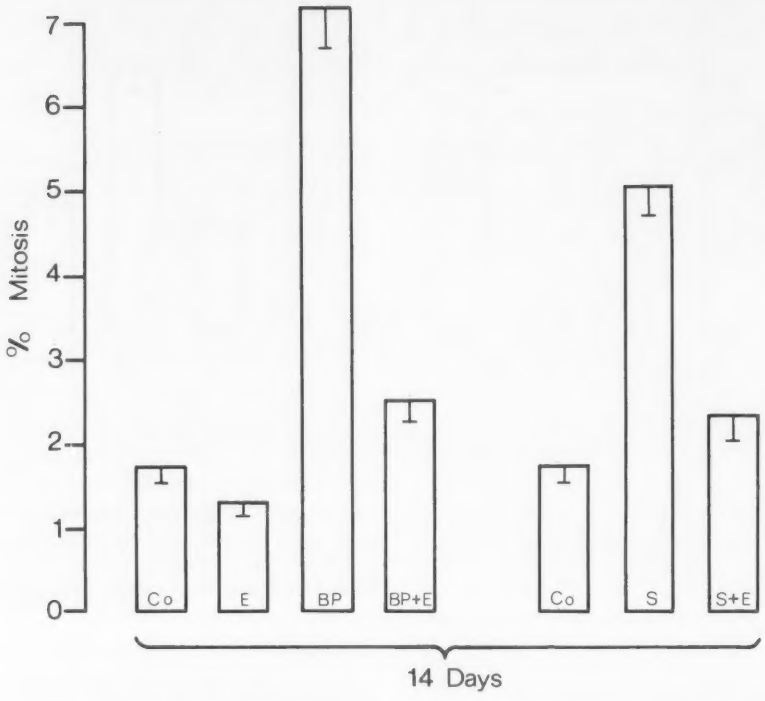

FIGURE 3.—Prevention of benzpyrene (BP)- and cigarette smoke condensate (S)-induced mitotic stimulation of tracheal epithelium by etretinate (E). Co = control medium.

area and has developed a ciliary apparatus and formed mucin-secreting goblet cells. Occasionally, reserve cells can be seen between the superficial ciliated epithelium and the basement membrane.

Exposure to the aromatic retinoid etretinate does not alter this basic pattern, and the epithelium is indistinguishable from that of the control explants.

In contrast, 3,4-benzpyrene has a profound effect. It stimulates the proliferation of the epithelium, often resulting in a multilayered, often-folded epithelium. At the same time, it has greatly reduced the number of goblet cells and has a deleterious effect on the cilia which have become clumped. In some tracheas, the epithelium shows squamous metaplasia with the formation of strands of keratin.

In explants exposed to cigarette smoke condensate, a similar although somewhat weaker effect can be observed: stimulation of cell proliferation associated with a loss of goblet cells and clumping of cilia.

As in the lung, the changes persist after transfer to control medium. Addition of the retinoid inhibits the benzpyrene- or smoke condensate-induced changes in a striking manner. If combined with either compound, etretinate inhibits the increase in cell proliferation and prevents the loss of goblet cells and the damage to the cilia; if applied after them, it reduces cell proliferation and restores the ciliary apparatus and the formation of goblet cells. Such epithelium closely resembles that of the controls.

The mitotic counts show (figs 3 and 4) that the percent of mitosis is increased over fourfold by 3,4-benzpyrene and nearly threefold by the cigarette smoke condensate. The retinoid alone does not affect the normal mitotic rate, but if combined with benzpyrene or the smoke condensate it reduces the increase to near control levels.

In explants pretreated with either carcinogen or smoke condensate and transferred to control medium, the rate of cell division amounts to six and

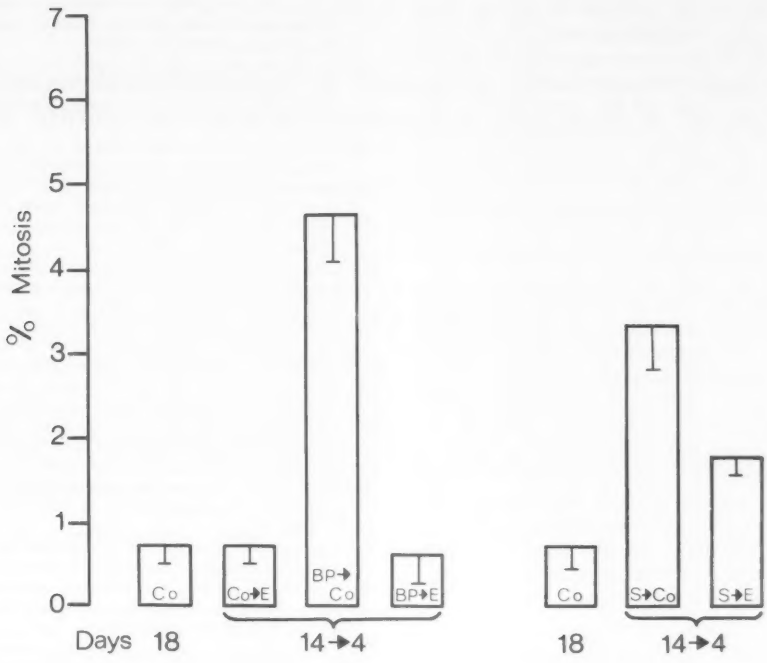

FIGURE 4.—Reversal of benzpyrene (BP)- and cigarette smoke condensate (S)-induced mitotic stimulation of tracheal epithelium by etretinate (E). Co = control medium.

four times the control value, respectively. In both sets, administration of etretinate for 4 days reverses the increase and reduces it significantly.

DISCUSSION

In both fetal mouse lung and neonatal rat trachea, 3,4-benzpyrene and cigarette smoke condensate stimulate epithelial cell proliferation and increase its mitotic rate. The activity of the smoke condensate which contains small quantities of benzpyrene and other carcinogenic and noncarcinogenic hydrocarbons is somewhat lower than that of benzpyrene. In both organs, the increase of epithelial cell replication is accompanied by a loss of secretory activity. In the bronchial epithelium, hyperplastic foci are characterized by crowded basal cells with the loss of the superficial secretory cells; in the trachea, the number of goblet cells is greatly reduced and the cilia are severely damaged. Similar epithelial growth promotion associated with metaplastic changes has been described for benzpyrene- and smoke condensate-treated human fetal lung and mouse tracheal epithelium in vitro (21–23) and for hamster tracheal cultures exposed to benzpyrene (24,25).

Administration of the aromatic retinoid etretinate has a profound effect both on cell replication and differentiation. If combined with benzpyrene or smoke condensate, it inhibits the increase in cell proliferation, maintains the secretory activity of the bronchial epithelium, and prevents the loss of goblet cells and cilia in the trachea. If added following exposure to the carcinogens, it reverses epithelial hyperplasia and restores the secretory activity of the bronchial epithelium as well as goblet cell and cilia formation.

Under both conditions, the action of the retinoid results in architecture and cytology which closely approximate those of the control tissues.

Similar effects of natural and synthetic vitamin A compounds have been reported for the same system. In hamster trachea grown in organ culture, vitamin A inhibited or reversed benzpyrene-induced hyperplasia and metaplasia (24,25), and in vitamin A-deficient tracheal cultures retinoids prevented squamous metaplasia (26).

The mechanism of action involved in the anticar-

cinogenic activity of the retinoid is not yet fully understood. It is now recognized that retinoids play an important role in the synthesis of glycosaminoglycans and are implicated in the transfer of sugar moieties to glycoproteins (27,28). In both lung and trachea, the restoration of the secretory function is associated with an inhibition of the carcinogen-induced cell replication. It is possible that these two events are linked and that a diminished proliferation rate allows the specialized cell functions to be reestablished. A similar inverse relationship between epithelial DNA synthesis and the reversal of squamous metaplasia was seen in prostatic epithelium exposed to methylcholanthrene and retinoids (29).

However, the link between cell proliferation and differentiation found in secretory epithelia is not seen in all tissues. In DMBA-induced skin papillomas treated with the same aromatic retinoid, the regression is not associated with diminished basal cell replication (30) but can be attributed to the deposition of glycosaminoglycans which disrupt the continuity of the hyperplastic keratinized cell layers and cause their breakdown (31,32). Thus, the mechanisms by which retinoids affect target organs appear to be specific for each given tissue.

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Tubulin-Dependent Biochemical Assay for the Antineoplastic Agent Taxol and Application to Measurement of the Drug in Serum[1]

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SUMMARY

A biochemical assay for taxol with sensitivity to 0.1 μM has been developed. Taxol-dependent formation of tubulin polymers occurs at 37°C in 1.0 M glutamate in the absence of GTP. These polymers are cold-stable and hydrolyze GTP at 0°C, whereas tubulin alone will not hydrolyze the nucleotide in the cold. This assay has been used to follow rabbit serum levels of taxol injected iv. Although the drug appears to be almost totally protein-bound, it is nevertheless rapidly cleared from serum. The apparent α-phase and β-phase half-lives after iv bolus administration in one rabbit are 2.7 and 42 mins, respectively.

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Taxol, a natural product derived from *Taxus brevifolia* (1), is a promising new antineoplastic agent that soon should enter clinical trials. The target of the drug appears to be the microtubule, but taxol has a unique mechanism of action. Rather than inhibiting tubulin polymerization, it markedly enhances the reaction (2–5). Moreover, microtubules formed in the presence of taxol are unusually stable: they remain intact at low temperatures, in the presence of Ca^{2+}, and after dilution, conditions that normally lead to the rapid disaggregation of microtubules (2–5).

The effect of taxol on tubulin-dependent GTP hydrolysis is highly dependent on reaction conditions (4,5). In some cases no substantial effect on the reaction is observed (4,5), but under other conditions GTP hydrolysis is dependent on the presence of taxol (5). This taxol-dependent GTP hydrolysis is associated with tubulin polymerization, with the drug inducing both polymerization and nucleotide hydrolysis under conditions where they otherwise do not occur (5). In preliminary studies we had found that micromolar taxol concentrations, substoichiometric to the tubulin concentration we were using, resulted in definite stimulation of GTP hydrolysis. Since this approached anticipated serum concentrations of the drug (M. C. Lowe, personal communication), we decided to explore its stimulation of tubulin-dependent GTP hydrolysis as a potential assay for taxol.

MATERIALS AND METHODS

Highly purified calf brain tubulin was prepared as described previously (5,6), including gel filtration chromatography to remove unbound nucleotide. Taxol was obtained from the Natural Products Branch, Division of Cancer Treatment, National Cancer Institute, and was dissolved in dimethylsulfoxide. Nonradioactive GTP from Sigma Chemical Co was repurified by triethylammonium bicarbonate gradient chromatography on DEAE-Sephadex A-25. Stock solutions of monosodium glutamate, purchased from Grand Island Biological Co, were adjusted to pH 6.6 with HCl. Polyethylenimine cellulose thin-layer sheets were from Brinkmann. High specific activity [α-^{32}P]GTP (initially

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2000–3000 curies/mmol) was purchased from Amersham.

GTP hydrolysis was determined by following the formation of [α-^{32}P]GDP from [α-^{32}P]GTP by thin-layer chromatography in 1.0 M KH_2PO_4 and autoradiography (6). Specific conditions are described in the individual experiments; all assay mixtures contained 10% dimethylsulfoxide. All assays were performed in triplicate. Reactions were stopped by adding 20 μl of reaction mixture to 40 μl of 25% acetic acid. Of this mixture, 10 μl was spotted in triplicate on polyethylenimine cellulose thin-layer chromatography sheets (all experimental points are thus the average of nine values), and chromatography was begun while the spots were still wet.

TABLE 1.—Effect of preincubating taxol and tubulin at 37°C on subsequent GTP hydrolysis at 0°C*

	% GTP hydrolyzed	
	Not preincubated	Preincubated
No taxol	3.7	3.6
+ 1 μM taxol	16.9	42.7

*Each 45-μl reaction mixture contained 10 μg of tubulin, 50 μmols of glutamate, 50 nmols of $MgCl_2$, and indicated concentration of taxol (taxol concentration applies, however, to final reaction volume of 50 μl). If indicated, reaction mixtures were preincubated for 1 hr at 37°C and then chilled on ice. A 5-μl sample containing 0.1 μM [α-^{32}P]GTP was added to each reaction mixture. Samples were left on ice for 18 hrs and then analyzed for GTP hydrolysis.

RESULTS AND DISCUSSION

Our initial efforts were directed at maximizing the sensitivity of the assay for taxol and minimizing the baseline reaction. The best results were obtained using 1.0 M glutamate, 1 mM $MgCl_2$, and 0.2 mg/ml of tubulin and a 0°C incubation.

We have found that 1.0 M glutamate induces a GTP-dependent, temperature-dependent polymerization of purified tubulin coupled initially to stoichiometric GTP hydrolysis (6). Previously we had observed no GTP hydrolysis at 0°C, but easily detectable GTP hydrolysis occurred at 37°C with high specific activity [α-^{32}P]GTP at nucleotide concentrations unable to support tubulin polymerization (6). With the addition of taxol, tubulin polymerization will occur at 37°C without GTP or at 0°C provided GTP is present (5). This taxol-dependent polymerization in 1.0 M glutamate at 0°C is associated with GTP hydrolysis [unpublished observations; (5)]. Measurement of tubulin-dependent GTP hydrolysis at 0°C in 1.0 M glutamate therefore provided a useful assay system for taxol, since there is a minimal reaction in the absence of the drug. Finally, we found that tubulin-dependent GTP hydrolysis in 1.0 M glutamate is not affected by dimethylsulfoxide concentrations as high as 30% (7). This is important since taxol is poorly soluble in water but readily soluble in dimethylsulfoxide.

Taxol stimulation of tubulin-dependent GTP hydrolysis at 0°C is significantly enhanced by preincubating taxol and tubulin at 37°C in the absence of GTP (table 1). Since this probably results in the formation of stable polymerization products prior to the addition of GTP (2,4,5), it is most likely these tubulin polymers that are responsible for most of the hydrolytic reaction. Because of the significant enhancement of the taxol-stimulated reaction, with no effect on the baseline, this drug-tubulin preincubation at 37°C was performed in all experiments presented here. With the prolonged 0°C incubation of this experiment, there was definite, although slight, hydrolysis of GTP by tubulin alone.

Sensitivity for taxol could also be enhanced if high specific activity [α-^{32}P]GTP was used (fig 1). The difference between 0.01 and 0.1 μM GTP is not substantial and probably not significant, but at these nucleotide concentrations sensitivity for taxol is substantially greater than at 10 μM GTP. Sensitivity is also enhanced at the lower GTP concentrations by the greater range of the reaction, the plateau occurring when > 90% of the GTP is hydrolyzed rather than 40% as occurs with 10 μM nucleotide.

Figure 2 demonstrates the reproducibility of the assay, using 0.01 μM GTP. Three separate experiments were performed, with all experimental points representing an average of nine values. The inset demonstrates that sensitivity was at least as low as 0.1 μM taxol in two of these experiments and 0.2 μM taxol in the third. Figure 3A shows an autoradiogram demonstrating the hydrolysis of GTP observed with 1 μM taxol.

We next examined the suitability of this assay for the determination of taxol levels in serum. Since the phosphatase activity of serum was substantial (fig 3A), methods of treating serum were evaluated for effectiveness in eliminating these enzymes while preserving the taxol content of serum. In these experiments exogenous taxol was added to human serum at 10 μM, and 10% treated serum was used in assays for a final nominal taxol concentration of 1 μM.

Heat and perchlorate treatment of serum were both effective in eliminating its phosphatase activity, but exogenously added taxol was also lost (data not presented). Similar results were obtained with ultrafiltration of serum (fig 3B, table 2), suggesting that taxol is largely bound by serum proteins.

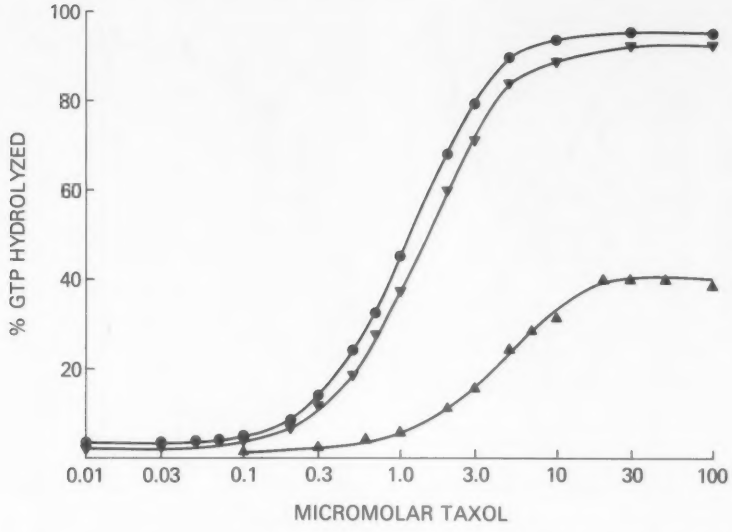

FIGURE 1.—Effect of increasing specific activity of [α-^{32}P]GTP on taxol-dependent stimulation of GTP hydrolysis by tubulin. Each 45-μl reaction mixture contained 10 μg of tubulin, 50 μmols of glutamate, 50 nmols of $MgCl_2$, and indicated concentration of taxol (taxol concentrations apply, however, to final reaction volume of 50 μl). Reaction mixtures were preincubated for 1 hr at 37°C and then chilled on ice. A 5-μl sample containing [α-^{32}P]GTP of varying specific activity was added to each reaction mixture for final nucleotide concentrations of 10 μM (▲), 0.1 μM (▼), or 0.01 μM (●). Samples were left on ice for 18 hrs and then analyzed for GTP hydrolysis. Each experimental point was performed in triplicate, and each of these was analyzed in triplicate. Average of 9 values obtained is presented. Ranges obtained are similar to those presented in fig 2. Average % GTP hydrolyzed in absence of taxol was as follows: at 10 μM GTP, 0.9%; at 0.1 μM GTP, 2.4%; and at 0.01 μM GTP, 3.6%.

The most effective treatment we have thus far found consists of precipitation of serum proteins with four parts dimethylsulfoxide and their removal by centrifugation, followed by lyophilization of the supernatant and resuspension of the residuum in a volume of dimethylsulfoxide equivalent to the original serum volume (fig 3C, table 2). This essentially eliminates serum phosphatase activity and leads to the recovery of > 75% of the added taxol (average of four experiments was 80% recovery), presumably because of the high solubility of the drug in dimethylsulfoxide.

To demonstrate the usefulness of this assay for the determination of taxol levels in clinical specimens, the serum extraction method was used in a preliminary experiment to follow the pharmacokinetics of taxol in the rabbit, except that the lyophilized residua were dissolved in one half the original serum volumes to enhance sensitivity (fig 4). In this experiment, we attempted to minimize the effect of incomplete extraction of taxol: the standard curve (fig 4, inset) was generated from samples of baseline rabbit serum containing known concentrations of taxol that were extracted in the same way as the serum samples obtained after injection of the drug.

Figure 4 demonstrates that taxol, after rapid iv injection in a rabbit at a dose of 8.5 mg/kg, is rapidly cleared from the serum.[4] Assuming a two-compartment open model (8), its apparent α-phase half-life was 2.7 mins, and its β-phase half-life was 42 mins. At 2 hrs the serum level was < 3% of that found at the initial 1-min time point. An ultrafiltrate was also prepared from the serum sample obtained 5 mins after drug injection. This contained about 8% of the taxol found in the serum extract, comparable to the result shown in table 2 with exogenously added taxol (data not presented).

In summary, we have developed a biochemical assay for taxol with sensitivity to 0.1 μM. This assay exploits the ability of taxol to induce tubulin to form cold-resistant polymers that hydrolyze GTP at 0°C. We have found that taxol is almost totally protein-bound but is nevertheless cleared rapidly from se-

[4]For comparison, an optimal therapeutic dose with mouse B16 melanocarcinoma was 5 mg/kg ip daily for 9 days (M. C. Lowe, personal communication).

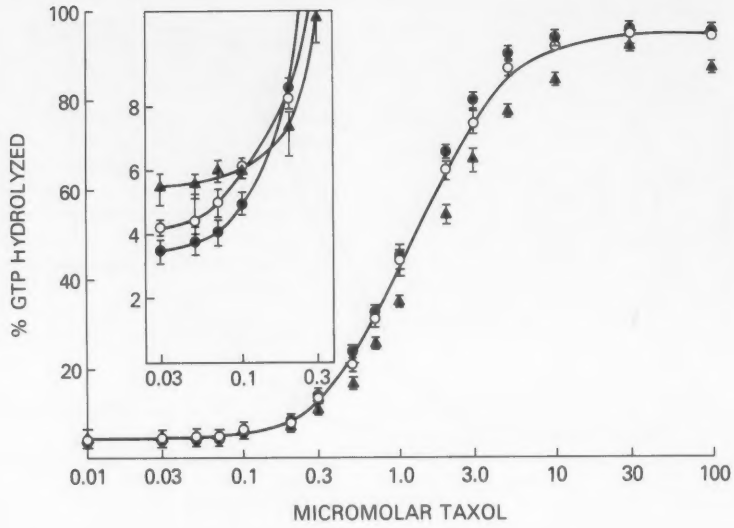

FIGURE 2.—Experimental reproducibility of taxol-dependent stimulation of GTP hydrolysis by tubulin at 0.01 μM GTP. Experimental conditions were identical to those described in fig 1, except that all 3 experiments were performed with 0.01 μM [α-^{32}P]GTP. Average % GTP hydrolyzed in absence of taxol was as follows: 1st experiment (●), 3.6% (range, 3.2%–3.9%); 2nd experiment (▲), 5.1% (range, 4.8%–5.3%); and 3rd experiment (○), 3.9% (range, 3.5%–4.2%). Inset presents data from 0.03–0.3 μM taxol on an expanded scale.

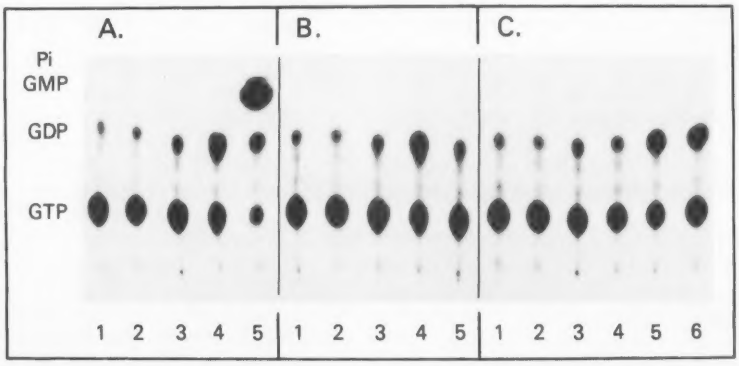

FIGURE 3.—Autoradiograms of reaction mixtures used to study effect of taxol on the GTPase activity of tubulin. Reaction conditions were identical to those described in table 2, with all mixtures containing GTP, glutamate, $MgCl_2$, and dimethylsulfoxide. A) Effects of tubulin, taxol, and serum. Additions to reaction mixtures were as follows: 1 = no addition; 2 = 1 μM taxol; 3 = 10 μg of tubulin; 4 = 10 μg of tubulin and 1 μM taxol; and 5 = 5 μl of human serum. B) Effect of serum ultrafiltration. Additions to reaction mixtures were as follows: 1 = no addition; 2 = 5 μl of serum ultrafiltrate; 3 = 5 μl of serum ultrafiltrate and 10 μg of tubulin; 4 = 10 μg of tubulin and 1 μM taxol; and 5 = 10 μg of tubulin and 5 μl of an ultrafiltrate of serum containing 10 μM taxol. C) Effect of dimethylsulfoxide extraction of serum. Additions to reaction mixtures were as follows: 1 = no addition; 2 = 5 μl of serum extract; 3 = 10 μg of tubulin; 4 = 10 μg of tubulin and 5 μl of serum extract; 5 = 10 μg of tubulin and 1 μM taxol; and 6 = 10 μg of tubulin and 5 μl of an extract of serum containing 10 μM taxol, with extract having same volume as original serum.

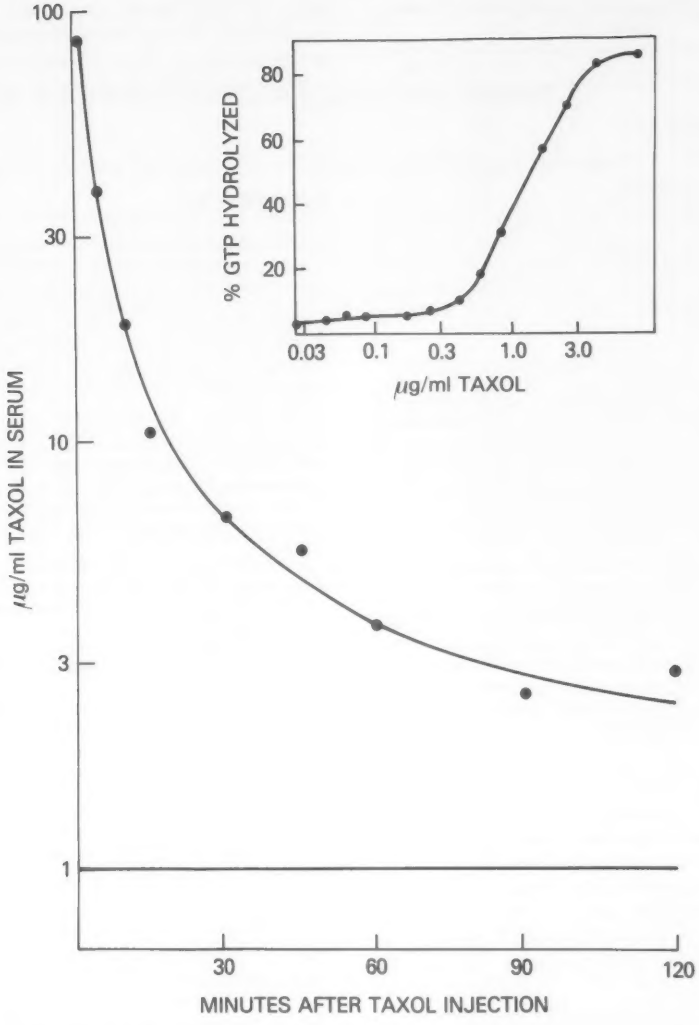

FIGURE 4.—Taxol pharmacokinetics following bolus iv injection. A 3.5-kg rabbit was anesthetized by iv injection of 180 mg of sodium pentobarbital, and femoral artery and vein were cannulated. A total of 1.75 ml of dimethylsulfoxide containing 17 mg of taxol/ml (0.02 M) was injected iv over 30 secs, and 1-ml blood samples were drawn at indicated times. Serum was prepared from blood samples, mixed with 4 parts dimethylsulfoxide, and centrifuged for 5 mins at 20,000 × *g*. Protein pellets were discarded, and 0.75 ml of each supernatant was lyophilized. Residua were dissolved in 0.075 ml of dimethylsulfoxide, and resulting solutions were used to determine the taxol content of serum. Each 45-μl reaction mixture contained 10 μg of tubulin, 50 μmols of glutamate, 50 nmols of $MgCl_2$, and 5 μl of serum extract or 5 μl of a mixture of an extract of baseline serum and extract of serum obtained at indicated time. Reaction mixtures were preincubated for 1 hr at 37°C and then chilled on ice. A 5-μl sample containing 0.1 μM [α-^{32}P]GTP was added to each reaction mixture. Samples were left on ice for 18 hrs and analyzed for GTP hydrolysis. Data in this figure are presented in μg/ml rather than as molar concentrations because drug appears to be largely protein-bound (0.85 μg/ml is equivalent to 1 μM). Minimum detectable level of taxol was about 1 μg/ml. Inset: standard curve used to generate data of main portion of figure. Known amounts of taxol were added to aliquots of rabbit serum that were extracted and assayed as described above. Values on abscissa refer to original concentrations in serum.

TABLE 2.—Recovery of exogenously added taxol from human serum*

	% GTP hydrolyzed
+ Tubulin only	3.1
+ Tubulin + 0.7 μM taxol	22.3
+ Tubulin + 1.0 μM taxol	34.1
+ Serum ultrafiltrate	0.3
+ Tubulin + serum ultrafiltrate	3.4
+ Tubulin + ultrafiltrate of serum containing taxol (final concentration nominally 1.0 μM)	3.8
+ Serum extract	0.8
+ Tubulin + serum extract	5.4
+ Tubulin + extract of serum containing taxol (final concentration nominally 1.0 μM)	25.3

*Each 45 μl of reaction mixture contained 50 μmols of glutamate, 50 nmols of $MgCl_2$, and the following, as indicated: 10 μg of tubulin, taxol as indicated (concentrations applying to final reaction volume of 50 μl), 5 μl of serum ultrafiltrate, 5 μl of ultrafiltrate of serum containing 10 μM taxol, 5 μl of extracted serum, and 5 μl of extract of serum containing 10 μM taxol. Reaction mixtures were preincubated for 1 hr at 37°C and then chilled on ice. A 5-μl sample containing 0.1 μM [α-^{32}P]GTP was added to each reaction mixture. Samples were left on ice for 18 hrs and analyzed for GTP hydrolysis. Serum ultrafiltrates were prepared with Amicon MPS-1 micropartition filtration devices. Extracted serum was prepared by mixing 0.5 ml of serum with 2.0 ml of dimethylsulfoxide. Precipitate was removed by centrifugation at 20,000 × *g*, and 1.6 ml of the supernatant was lyophilized. Residuum was dissolved in 0.32 ml of dimethylsulfoxide. Serum containing taxol was prepared by mixing 0.495 ml of serum with 5 μl of 1 mM taxol. Ultrafiltration and extraction were performed as described above.

rum. It is obviously important to determine the mechanism of this rapid clearance for the appropriate design of chemotherapeutic regimens. Such studies are currently being planned in our laboratory.

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Workshop on Kaposi's Sarcoma: Meeting Report

William D. DeWys,[1,*] James Curran,[2] Werner Henle,[3] and Gerhard Johnson[4]

On July 3, 1981, investigators from the Centers for Disease Control (CDC) reported a clustering of cases of Kaposi's sarcoma (KS) in homosexual men in New York City and California (1). KS is a rare tumor in North America and Europe and usually occurs in aged men (mean age, 63 years). The 26 cases reported occurred in younger men (range, 26–51 years; mean, 39), and seven cases also had serious infections including *Pneumocystis carinii* pneumonia (PCP), CNS toxoplasmosis, extensive candidiasis, and cryptococcal meningitis. All patients tested had serologic evidence of past or present cytomegalovirus (CMV) infection.

Based on this report and a subsequent followup (2), the Division of Cancer Treatment and the Division of Cancer Cause and Prevention of the National Cancer Institute (NCI) and the Task Force on Kaposi's Sarcoma and Opportunistic Infections of the CDC sponsored a Workshop on Kaposi's Sarcoma on September 15, 1981, at the National Institutes of Health.

At the workshop, **J. Curran,** CDC, reviewed the series of cases reported to the CDC to date (1–3), which included 55 cases of KS, 49 cases of PCP, and 11 cases of both diseases, either concurrently or sequentially. All cases were in men, 94% of whom were homosexual or bisexual. Eighty percent of these patients were between 25 and 45 years of age. The classic dark blue or violaceous plaques or nodules on the skin or mucous membranes were present in most cases, but other frequent presenting signs and symptoms included lymphadenopathy, fever, diarrhea, and weight loss. More than 85% of the men resided in New York City, Los Angeles, or San Francisco, which correlated with known concentrations of homosexual men. Since one would expect < 100 cases of KS in the US per year, these observed cases in homosexual men suggest an epidemic of this disease in this population. Although preliminary epidemiologic observations suggest a correlation of KS with sexually transmitted diseases, increased numbers of sexual partners, and use of nitrite inhalants, investigators from CDC have initiated a detailed case-control epidemiologic study to more clearly identify and delineate these risk factors.

Ziegler, Vogel, and **Templeton** reviewed the natural history of nonepidemic KS. Four clinical categories have been described. The *nodular* form generally is chronic, indolent, and associated with some degree of edema, appearing in most cases on the extremities; persons with nodular KS may not seek medical attention unless the nodules ulcerate, become somehow incapacitating, or begin to invade deeper tissues. This is the predominant form in elderly men. The *florid* form features an exophytic sarcomatous transformation of a nodule which then becomes locally aggressive and invasive. In the yet more serious *infiltrative* form of KS, the tumor penetrates deeply and very often will involve underlying bone. The florid and infiltrative forms are seen predominantly and endemically in equatorial Africa. Finally, the *generalized* form has been termed lymphadenopathic because it often presents with multiple lymph node enlargements; the disease is primarily associated with mucosal lesions, often becoming disseminated through the lungs and gastrointestinal tract, and the prognosis is poor because of the generalized aggressive nature of this form of KS. This is the form commonly seen in the current US epidemic and in African children and young adults.

The nonendemic form of KS occurs predominantly in older males, with an apparent predilection for persons of Jewish or Italian ancestry. The endemic incidence is 100-fold greater than the nonendemic incidence. An increased incidence of KS occurs in recipients of renal allografts, a median of 16 months after allografting. If immunosuppression is withdrawn, some Kaposi lesions regress.

KS may have several patterns of dissemination. The most common pattern is regional development of multiple cutaneous and subcutaneous nodules. Spread to regional nodes may occur, but this must be differentiated from inflammatory lymphadenopathy secondary to tumor ulceration and infection. Involvement of multiple mucosal surfaces through-

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TABLE 1.—Results of treatment in Ugandan patients with KS

Drug regimen	No. of evaluable patients	Response		
		Complete	Partial	None
Dactinomycin	12	2	7	3
Cyclophosphamide	10	1	0	9
Dactinomycin + vincristine	14	10	3	1
Dactinomycin + vincristine + dacarbazine	32	30	1	1
Data compiled from the literature for other agents				
BCNU	21	4	5	12
Bleomycin	10	0	6	4
Dacarbazine	10	0	5	5
MOPP	5	0	0	5
Daunorubicin	3	0	0	3
ICRF-159	47	10	17	20

out the airway and gastrointestinal tract is a unique feature of this disease. Finally, visceral metastases, especially to lung, may develop. Based on these patterns, evaluation of extent of disease in KS may require lymph node biopsy, laryngoscopy, and gastrointestinal endoscopy in addition to the usual clinical examinations, radiographs, and scans.

G. Giraldo described studies on the possible role of CMV in KS. Antibody titers to CMV were higher in KS patients compared to controls in studies in the US and Italy, but titers in African patients did not differ significantly from African controls (African controls also had elevated titers). In recent work, Giraldo (4) has been able to identify CMV DNA and gene products in about one third of KS tissues using several techniques. **L. Drew** reported high titers of IgG antibodies to CMV in homosexual men. **Friedman-Kien** reported decreased helper T lymphocytes and increased T suppressor cells in homosexual patients with KS, as studied with monoclonal antibodies. However, studies of T-cell function were not reported. HLA typing of KS patients by **P. Rubenstein** showed 61% having the DR-5 antigen, compared with 19% in a control population.

Ziegler reviewed the results of treatment in Ugandan patients as shown in table 1.

Laubenstein reported on a trial of doxorubicin, bleomycin, and vinblastine, which has produced partial remissions in all nine patients treated so far. KS is also radiosensitive, and wide-field radiation therapy merits further evaluation.

After the above presentations and discussions, the workshop participants divided into three groups to develop recommendations for future studies.

Epidemiology

The group considering epidemiology, chaired by J. Curran, reported that a national case/control study of KS and/or PCP among homosexual men will be conducted by CDC, and they stated that it would be desirable to have laboratory studies coordinated with this study. They recommended national retrospective and prospective active surveillance of KS and PCP in the US. The following suggestions for future studies were also made:

(*a*) Definition of the magnitude and nature of the "immunosuppressive syndrome" among homosexual men. How large is the group at risk for KS and/or PCP?

(*b*) Examination of recent past and future cancer incidence data to detect an excess of other cancers among homosexual men (or single men) especially from geographic areas such as New York City and San Francisco. Special attention should be directed to tumors associated with immunosuppression.

(*c*) Use of a *retrospective cohort* approach to study illness incidence among homosexual men. The participants in the CDC and New York Blood Center hepatitis B vaccine trials have been characterized by antigen and behavioral studies and could be contacted for followup.

(*d*) An epidemiologic study of elderly men with Kaposi's sarcoma, examining sex preference, marital status, exposure to various nitrites, etc.

(*e*) Prospective followup of immunosuppressed homosexual men at risk for developing KS or PCP.

Virology-Etiology

The session on virology-etiology, chaired by **W. Henle** concluded that at present, CMV is the leading candidate for an etiologic role in KS; recommendations for future studies are heavily oriented toward this virus. While evidence for a relationship between CMV and KS has been steadily mounting,

due largely to the efforts of Giraldo and his associates, there are some as yet unresolved discrepancies, such as the difference in antibody responses to CMV between white KS patients (elevated titers) and African patients (not different from controls) and the absence of CMV "fingerprints" in some of the KS biopsies that have been tested. It is essential, therefore, to keep an open mind and to look for other viruses that might be associated with the tumor. The studies recommended would detect not only CMV but other viruses or viral products.

Of primary importance is the collection of appropriate, well-characterized specimens from KS patients and controls in a coordinated manner. A central depository from which qualified investigators could draw needed specimens would be most desirable. Thus, aliquots of specimens would be tested in several laboratories by possibly different techniques permitting comparisons and correlation of results. The specimens to be collected include biopsy material (frozen or fresh) and sera.

Biopsy material.—Frozen biopsy specimens are needed for the detection of viral nucleic acids by appropriate hybridization techniques, including in situ tests to identify the specific type of cells harboring viral genomes. Fresh biopsy specimens are needed for initiation of cultures, making of touch preparations (which after fixation can be stored frozen), and transplantation to nude (athymic) mice to separate the malignant (growing) from normal (not growing) cells. The materials should be examined for the presence (or emergence) of viruses, viral antigens, or viral nucleic acids by pertinent techniques: eg, virus isolation and identification; and immunofluorescence (IF) by the indirect and anti-C′ techniques using virus-specific reference sera and virus-specific nucleic acid hybridization tests. It will be essential to obtain and test control specimens also; ie, biopsy material from apparently normal tissue adjacent to KS lesions. When found histologically free of KS, these specimens should be tested in parallel with the KS specimens of the same patient to differentiate between strictly tumor-associated virus or viral products and persistent latent, perhaps activated, infections in anatomic regions serving as a natural habitat for the virus.

Sera.—These should be collected from:

(*a*) KS patients before initiation of therapy and serially thereafter at intervals of 3–4 months or more frequently based on clinical events.
(*b*) Homosexual men without KS but having a lifestyle similar to that of KS patients. If CMV antibody-negative individuals are identified, they should be followed prospectively, if possible, to determine their immune responses to primary CMV infections when they occur.
(*c*) Healthy controls matched for age, sex, race, and socioeconomic status.
(*d*) Patients with primary CMV infections and patients with recurrent CMV infections.

Several different serologic tests are being used for measuring antibodies to CMV. Of these, IF has the advantage of visually differentiating between various distinct virus-coded antigens by their cellular localization and the morphology of staining. IF also can identify the immunoglobulin class of the reactive antibodies. Most other techniques require separation and a high degree of purification of CMV-coded antigens, which are not readily accomplished.

The preparation of cell smears for measuring antibody titers to various CMV-coded antigens by indirect or anti-C′ IF is presently cumbersome and not amenable to mass production, since it depends on cells grown and infected on coverslips. Efforts should be made to find cells that grow in suspension cultures and are capable of synthesizing the various CMV-coded antigens under specified conditions. Cell smears could then be made which should be uniform with respect to the number and distribution of antigen-containing cells.

The virus-specific antibody spectrum and titers should be determined for patients with primary or recurrent CMV infections, healthy or immunologically compromised carriers of the virus, and, of course, KS patients at various stages of the disease. There could conceivably be an antibody pattern characteristic of KS which could serve as a diagnostic aid. Also, long-term serologic followup studies on KS patients might reveal changes in the spectrum and titers of antibodies which might reflect the prognosis (decreases being favorable and increases unfavorable) as observed with the Epstein-Barr virus-specific antibody patterns in Burkitt's lymphoma and nasopharyngeal carcinoma.

If and when other candidate viruses for an etiologic relation to KS are found, they must be subjected to the same type of serodiagnostic and seroepidemiologic studies.

While the above approaches might implicate CMV or another virus in the etiology of KS, this alone would not suffice to explain completely the present epidemic of the disease in homosexual men. The key questions remain as to what causes the extreme immunodeficiency in the patients and what comes first, KS or the immunosuppression. Probably the immune deficiency precedes KS. The various drugs, such as amylnitrite and butylnitrite, which have been used excessively by many homosexual KS patients, should be tested in animals for their immunosuppressive, mutagenic, and oncogenic potential.

Aggressive chemotherapy and persistent infec-

tions, such as CMV, Epstein-Barr virus, hepatitis B, pneumocystis, and amebiasis, likely contribute to the final degree of immunologic incompetence. Careful evaluation of the immune reactivities of KS patients before therapy and continuing thereafter is considered essential. It was pointed out that in addition to identifying various types of leukocytes by monoclonal antibodies, their functional state must also be ascertained.

Treatment

The working group considering treatment of KS agreed on a general format for any prospective study. Components of the protocol would include eligibility, pathology confirmation, staging, immunologic evaluation, infection prophylaxis, and treatment.

Eligibility.—Although trials would be primarily designed to offer a systematic approach to the treatment of the aggressive form of KS in male homosexuals, all patients with a diagnosis of KS would be eligible for entry into such a study.

Pathology confirmation.—A Central Pathology Review Panel should be established to review the tissues obtained from all patients entering clinical trials.

Staging.—Patients may be staged according to the scheme:

Disease location	Disease activity*	
Local/regional	Indolent	Aggressive
Generalized	Indolent	Aggressive

*Designation of aggressive disease is to be based on tempo of disease and involvement beyond skin and mucosal surfaces.

The following staging evaluation was recommended:

(*a*) Laboratory studies: hemoglobin, leukocyte count, differential, platelet count, reticulocyte count, erythrocyte sedimentation rate, serum protein electrophoresis, quantitative immunoglobulins, immunoelectrophoresis, VRL, serum creatinine, uric acid, bilirubin, SGOT, alkaline phosphatase, electrolytes, and calcium and phosphorus.

(*b*) X-ray studies: chest X-ray, upper gastrointestinal series with small bowel follow-through, barium enema, computerized axial tomographic scan of abdomen and pelvis.

(*c*) Radioisotope scans: liver/spleen scan, bone scan if alkaline phosphatase of bone origin is elevated.

(*d*) Endoscopy: upper gastrointestinal endoscopy and colonoscopy (bronchoscopy should be performed if the chest X-ray is abnormal and there is reason to suspect infection).

Immunologic evaluation.—Delayed hypersensitivity should be evaluated with a battery of skin tests, including *Candida, Trichophyton,* mumps, and purified protein derivative. Additional immunologic studies are to be defined.

Infection prophylaxis.—In view of the immunosuppression and propensity to infection with *Pneumocystis carinii* and other opportunistic infections, all patients should be treated prophylactically with trimethoprim and sulfamethoxazole.

Treatment.—Treatment assignment should be based on the patient's clinical stage of disease as defined above. Possible regimens are based on cumulative experience with this disease and pilot studies currently in progress at New York University, the University of California at San Francisco, and Memorial Sloan-Kettering Cancer Center.

For both indolent and aggressive local/regional disease, patients may be treated with regional radiation therapy. For indolent generalized disease, patients may be treated with vinblastine, ICRF-187[5] or VP-16-213.[6] For aggressive generalized disease, combination chemotherapy[7] may be used.

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[5]Patients without liver abnormalities (normal bilirubin and negative computerized tomographic scan of the liver) can receive 2.5 g/m²/wk, iv; patients with liver involvement, 1.5 g/m²/wk, iv. Treatment would be given weekly for 4 wks followed by a 2-wk rest period. If moderate myelotoxicity is not observed, a 20% dose escalation can be used. Appropriate dosage reduction should be made for patients who develop cytopenias.

[6]Patients may receive 150 mg/m²/day, iv, for 3 days; cycles can be repeated every 3–4 wks.

[7]Dactinomycin (1.5 mg/m², iv) on Day 1, dacarbazine (750 mg/m², iv) on Day 1, and vincristine (2 mg, iv) on Days 1 and 15 can be given, as can doxorubicin (40 mg/m², iv) on Day 1, vinblastine (6 mg/m², iv) on Day 1, and bleomycin (15 units, iv) on Days 1 and 15. Patients who fail one regimen might cross over to the other combination.

The Epidemic of Acquired Immune Dysfunction in Homosexual Men and Its Sequelae—Opportunistic Infections, Kaposi's Sarcoma, and Other Malignancies: An Update and Interpretation

Arthur S. Levine[1,*]

Since the original report of Gottlieb et al (1) on five cases of *Pneumocystis carinii* pneumonia (PCP) in young homosexual men from California, the report 1 month later of 26 cases of Kaposi's sarcoma (KS) occurring in a similar population (2), and the workshop held at the National Institutes of Health in September of 1981 (*see* companion report), much new information has become available. The following summary of that new information is abstracted from a second workshop (held at the Centers for Disease Control [CDC], March 3, 1982), from recent reports in the literature or in press, and from personal communications.

INCIDENCE AND SURVIVAL

At the time of this writing, 300 cases of the new syndrome have been reported, 3% of which were diagnosed in 1979, 11% in 1980, and 86% since January 1981. Of these 300 patients, 135 have had PCP; 115, KS; 28, both diseases; and 22, other opportunistic infections (3,4). The mortality rate so far has been 40%; however, > 70% of patients with the earliest diagnosed cases have died, predicting that the ultimate mortality will be staggering. Eighty-five percent of the 300 patients have been < 45 years old, with a median of 35.

Of these 300 patients, 290 have been men. Of these 290 men, 242 have been homosexual or bisexual, 30 have been heterosexual, and 18 have been men with an unknown sexual preference. Ten of the 300 patients have been women (predominately heterosexual). Of the 300 patients, 52% have been from New York City, 22% from California, 23% from 19 other states, and 3% from six foreign countries (a number of these foreign patients had recently been to the United States) (3).[2] Of interest are a cluster of 11 cases reported from Haiti and a cluster within a prison population. On the average, one new case of this syndrome is being reported each day. Considering all of the epidemiologic observations together, a growing accumulation of risk factors is suggested rather than the spread of disease in the classic sense of contagion.

Forty-five percent of the 242 homosexual male patients have developed KS; 97% of all KS cases in this epidemic have occurred in homosexual males. Seventy-three percent of the PCP cases have occurred in homosexual males, and 38% of the homosexual male patients have developed this infection. Only 12% of the heterosexual male and the female patients have developed KS, but 72% of this group have developed PCP. Seventy-five percent of the homosexual male patients have been white, with the rest evenly divided between black and Hispanic; only 20% of the heterosexual male and the female patients have been white. As with the homosexual male patients, the heterosexual male and female patients have been concentrated in New York and California (3).

RISK FACTORS

The CDC Task Force has compared homosexual male patients with clinically normal homosexual and heterosexual male controls. These controls have been matched to the patients for age, race, and city of residence. Among homosexual males, a history of gonorrhea, hepatitis B, amebiasis, and/or cytomegalovirus (CMV) infection is very much more common than among heterosexual men; all of these infections, especially CMV, are virtually endemic in the homosexual male population. However, when patients with this syndrome are matched to homosexual controls, the only factors that are somewhat more prominent in the patients are the frequency of CMV isolation from the urine and a CMV antibody titer > 1:128; an elevated Epstein-Barr virus (EBV) titer; a positive serologic test for syphilis; and enterovirus isolation. KS cases tend to have elevated plasma IgG levels, and PCP cases elevated

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[2]Minutes of a workshop held at the CDC, Atlanta, GA, March 3, 1982 (unpublished).

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IgA levels, as compared with matched homosexual controls. The median number of lifetime male sexual partners for homosexual male patients is 1160 versus 524 for matched homosexual controls. Moreover, the number of "bathhouse partners" within the past year is increased in patients versus matched controls.[2]

Ninety-eight percent of the homosexual male patients have had a history of exposure to nitrites, mainly amyl or butyl nitrite inhalants, but no one nitrite preparation is over-reported, suggesting that whatever the involvement of nitrites in this syndrome, that involvement is not due to a contaminant.[2] A history of nitrite usage is just as prevalent in the matched male homosexual control population, with > 85% of apparently healthy male homosexuals in New York and California reporting such use during the past 5 years. Of the heterosexual male and the female patients, only 14% have had a history of nitrite exposure, not significantly different than the incidence in a matched heterosexual control population; however, 60% of the heterosexual male and female patients have had a history of intravenous drug abuse, mainly involving opiates (3). Nitrite use correlates closely with the number of sexual partners and even with specific homosexual practices, specific social events, and specific meeting places. Therefore, a very extensive case-control study will be required to sort out the relative contributions of nitrites and transmissible microbes; in fact, it may be impossible to identify independent risk factors. However, it is clear that the syndrome is occurring mainly in a particular subset of the homosexual male population, possibly but not exclusively defined by the number of sexual partners.

It is of great interest that nitrites are the only drug the use of which has sharply increased in the US during the 1970s.[2] Of interest also is the fact that the nonmedicinal-grade nitrites now in use were first manufactured in California and then shipped to New York City; at present they are virtually ubiquitous.

IMMUNE DYSFUNCTION

There is evidence that a very sizable number of apparently healthy homosexual men in the US, especially those residing in New York and California, now have an acquired immune dysfunction. Goedert et al (4) have reported that seven of eight unselected and clinically normal homosexual men with a history of nitrite usage had a low T lymphocyte helper/suppressor (T-H/S) ratio, with OKT8+ cells increased in number, as detected by monoclonal antibody, and OKT4+ cells reduced. In cases of the new syndrome, the T-H/S ratio has been about 0.7 versus 1.8 in controls (5–8).[2] The reduction in helper cell numbers is absolute, but the increase in suppressor cell numbers is usually relative (although some patients have had a modest absolute increase). In the study by Goedert et al, only one of seven unselected and clinically normal homosexual males without a history of nitrite usage had a low T-H/S ratio. Of all 15 clinically normal male homosexual volunteers studied by Goedert et al (4), 14 had high CMV titers, and there was no correlation between CMV titers and nitrite use or T-H/S ratios. Thus, CMV alone does not produce the inverted T-H/S ratio, but nitrites may. One can extrapolate from these findings to the prediction that tens of thousands of homosexual men may have the acquired immune dysfunction and be at risk for development of the clinical syndrome. Indeed, we very probably have seen only the tip of the iceberg.

In addition to the low T-H/S ratio, other findings in patients with this syndrome have included cutaneous anergy, lymphopenia (often profound), leukopenia, diminished in vitro lymphoproliferative responses to soluble antigens and phytohemagglutinin (two thirds of the cases have had this in vitro anergy), and diminished natural killer cell activity despite an increase in the number of OKT8+ cells (the latter has not been a consistent finding) (5,6). The number and function of B lymphocytes have been intact in patients, and often, as noted previously, the level of immunoglobulins has been increased. In fact, it is now apparent that some patients demonstrate autoimmune phenomena rather than KS or opportunistic infections (9).[2] These patients, with increased OKT4 positivity, have had manifestations of lupus, acquired autoimmune hemolytic anemia, or autoimmune thrombocytopenic purpura. It may be that this patient subset is evidencing polyclonal B cell activation early in the evolution of their syndrome.

The breadth of immune defects seen in this syndrome certainly explains the broad gap in host defenses against all classes of microbes that are associated with cellular immunity. The degree of immunodeficiency is extraordinary, and not approached even in patients treated chronically with cyclosporin A, cyclophosphamide, or steroids. Thus, this is the first documented epidemic of community-acquired immune dysfunction. The KS and opportunistic infections appear to be secondary to the acquired immune dysfunction, as occurs in renal transplant patients or patients with autoimmune disorders treated chronically with immunosuppressive agents. In these latter types of patients, when the immunosuppressive treatment is discontinued, the KS lesions may regress, again suggesting that

the immunodeficiency is primary and the malignancy and infections are secondary.

It is also of interest in this regard that renal transplant patients and patients treated chronically with immunosuppressive agents tend to have the generalized and aggressive form of KS, as do patients in the current epidemic (10). Moreover, KS patients, whatever the setting, have an increased incidence of lymphoma. Finally, the latency for appearance of KS in renal transplant patients is about 1½–5 years, which would appear to be consistent with the emerging picture of the present epidemic.

CYTOMEGALOVIRUS

As noted previously, CMV is endemic in the homosexual male population, being sexually transmitted via the urine and semen (11). Again, there is no evidence that primary infection is more common in patients with this new syndrome than in matched homosexual controls, but it is likely that once the patients have become immunodeficient, there is considerable reactivation of the CMV infection. CMV is itself immunosuppressive and can reduce the T-H/S ratio for up to 10 months after acute CMV mononucleosis in humans, as well as in the mouse model (11). However, as discussed above, the homosexual volunteers in the study by Goedert et al, with high CMV titers but no history of nitrite usage, did not show an altered T-H/S ratio. Therefore, it is probable that following the primary induction of immune dysfunction, a secondary CMV reactivation may lead to an increasingly profound immune deficiency as well as chronic antigenic challenge.

CMV is also a transforming virus in vitro; CMV DNA and antigens have been detected in a minority of KS tumors, and the virus has been isolated from a KS cell line (12). However, in the current epidemic, the CMV strains so far isolated show a diversity of restriction endonuclease patterns, and no new single mutant has emerged. While CMV has tropism for endothelial cells (the apparent cell of origin in KS), nothing precludes the possibility that CMV is merely a passenger in KS cells and not a primary oncogenic agent.

GENETICS

As noted in the accompanying report by DeWys et al, there is an association between the HLA-DR5 locus and the occurrence of KS in sporadic cases. This association is also suggested in the current epidemic, but there is no such association in patients who have PCP without KS (3).[2] Moreover, there may be an increased proportion of KS patients with Italian ancestry in the current epidemic, as is also true in sporadic cases, but again, these genetic associations are tentative at this time.

COURSE OF THE ILLNESS

It is now clear that this new syndrome reflects a broad spectrum of events, beginning with an asymptomatic acquired immune abnormality. In a small subset of the affected population, this abnormality may eventuate in autoimmune phenomena, but in most cases, the abnormality is an asymptomatic immune deficiency. Further along the spectrum is a prodromal syndrome with fever of unknown origin, diarrhea, weight loss, malaise, thrush, and wasting (5–7). In some respects, this prodromal syndrome is similar to that induced by a chronic viral infection or by graft-versus-host disease. Lymphadenopathy may or may not occur as an aspect of this prodrome; when it does, biopsy reveals reactive hyperplasia. The prodrome may last for weeks to months, and patients with lymphadenopathy may be stable for long periods. The most threatening end of the spectrum involves opportunistic infections and/or KS, and once any serious opportunistic infection has occurred, the course of these patients has been inexorably progressive (5–7).[2] The percentage of patients in whom the more benign events in this spectrum will lead to the more serious events is uncertain.

During the present epidemic, PCP has responded sluggishly and incompletely in most patients to TMP-SMZ and/or pentamidine. Moreover, there is a tendency for these patients to become severely leukopenic during treatment with TMP-SMZ even when they are given folates (6). Although TMP-SMZ prophylaxis has been recommended if laboratory findings suggestive of this syndrome are made, there is a question of whether the prophylactic regimen will be tolerated. With respect to other opportunistic infections, they reflect all classes of microbes associated with defective cellular immunity, including mycobacterial infections; interestingly, patients with mycobacterial infections have shown little or no granuloma formation (6).

As many as one third of KS patients in the epidemic appear to be developing other malignancies, as occurs with other KS patients, including the sporadic and endemic cases and the cases in renal transplant recipients or in patients receiving chronic immunosuppressive therapy (13). In the current epidemic, a number of patients have developed undifferentiated B cell lymphomas (apparently Burkitt's lymphoma). In three such patients, the tumors have been associated with EBV and one of the three with CMV as well (J. Ziegler, personal communication). Other tumors now being seen include squa-

mous cell carcinoma of the tongue and cloacogenic carcinomas. These tumor types have always occurred among male homosexuals, but the incidence appears to be significantly increased during the current epidemic (3).[2]

TREATMENT

To date, only about 15% of patients with generalized KS treated during this epidemic with chemotherapy (usually doxorubicin, bleomycin, and vinblastine) have had a complete response, as contrasted with the 90% complete response rate in equatorial Africa. Moreover, because of the tendency toward leukopenia in this new syndrome, these patients have not often been able to tolerate aggressive chemotherapy.[2]

In addition to chemotherapy and radiotherapy, or in lieu of them, KS cases have been treated in the current epidemic with thymosin, interferon, allogeneic bone marrow transplantation, and/or acyclovir (5–8).[2] Thymosin has yielded no tumor responses; there appear to be partial responses to interferon; acyclovir has produced no tumor responses; and the single marrow transplantation of which we are aware is too recent to evaluate. The CMV infection has also not responded to acyclovir, as might have been predicted from earlier studies of this virus. As noted above, the response of PCP to therapy has often been unsatisfactory.

INTERPRETATION

All of the facts now available regarding this epidemic suggest that the primary event is an acquired immune dysfunction, possibly triggered by nitrites since they are the only apparently new risk factor. CMV, other known transmissible agents, and homosexuality per se are not new (although the very high median number of partners is also of recent vintage). Moreover, in preliminary experiments at the CDC, nitrites appear to induce immunodeficiency in mice. In one earlier small study, nitrites were believed to induce lymphomas, and in the Ames test, nitrites are mutagenic. With respect to heterosexual male and female patients not using nitrites but reporting the intravenous abuse of other drugs, it should be noted that marijuana and opiates have also been reported to be immunosuppressive, with an opiate affinity for T lymphocyte receptors (14). As with all diseases, there clearly must be a genetic predisposition to various aspects of this syndrome, with most people having no adverse effects from the accumulated risk factors, some demonstrating autoimmune phenomena, and a sizable minority manifesting the immunodeficiency. Genetic factors probably also condition whether once the immunodeficiency has occurred, people at risk will develop KS, other malignancies, opportunistic infections, merely the prodrome, or no illness at all.

It is almost certain that CMV is not the primary immunosuppressive agent but that reactivation of CMV in the setting of immunodeficiency produces still further immunosuppression. A great diversity of other sexually transmitted viruses, protozoa, bacteria, and parasites are also known to suppress transiently one or another element of the immune system,[2] and once infections with these agents occur, they too may contribute to an increasingly profound immunosuppression. It is also notable that semen itself is immunosuppressive, and among homosexual men, the immunosuppressive factor(s) in the semen might readily gain access to the circulation through the friable rectal mucosa (15). "Donor" lymphocytes in the semen might gain access in the same way and establish a graft-versus-host disease, possibly reflected in the wasting prodrome. Any role that the semen plays, however, is probably secondary since, again, these homosexual practices are not new. It has also been observed that when antigens are presented orally to the gut-associated lymphoid tissue, suppressor cells may disseminate widely from the gut- to non-gut-associated lymphoid tissue, inducing a specific hyporesponsiveness to that antigen (16,17). This phenomenon may also have a role in the complex syndrome. Finally, as the illness evolves, malnutrition and cachexia undoubtedly produce further immune deficiency.

In the setting of repeated antigenic challenge and severe, persistent immunosuppression, KS and other malignancies may develop just as in patients who have received kidney transplants or who for other reasons are treated chronically with immunosuppressive agents. It seems likely that CMV is a passenger within KS cells rather than a primary oncogenic agent. It is also possible that a retrovirus is involved in these malignancies; moreover, as a consequence of intercourse with animals, an animal retrovirus might have been introduced in the homosexual population.

Once this syndrome has become clinically manifest, the various components have been difficult if not impossible to treat, and one may predict that an ultimate mortality rate approaching 70%–80% will be found. It is also apparent that conventional cancer chemotherapy and radiotherapeutic approaches will probably not be tolerable in this population and may not be effective. Where local radiotherapy and single-agent chemotherapy are plausible, an attempt at such treatments should be made, but what is undoubtedly required is a reconstitution of the immune system, again noting that

when immunosuppression is withdrawn from renal transplant patients with KS, the lesions may regress. This reconstitution may take the form of marrow transplantation or selective reconstitution with cellular immune elements, but clearly such an approach remains highly experimental and risky. It is also possible that monoclonal antibodies generated against potentially specific KS antigens might be of use therapeutically.

Finally, treatment of the KS and the opportunistic infections is usually unsuccessful at present, and prevention would appear to be the only practical remedy. Since nitrite exposure and promiscuity seem to be the only new elements contributing to this complex and tragic epidemic, both should be discouraged. Even if we cannot identify the contribution of these factors independently, we would soon learn whether such intervention yields a reduction in the number of reported cases.

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Treatment of Refractory Acute Nonlymphocytic Leukemia With a Combination of Pyrazofurin and Cytarabine[1]

Brief Reports

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SUMMARY

Six patients with refractory acute nonlymphocytic leukemia were treated with pyrazofurin (15 mg/m²) followed by cytarabine (100 mg/m²) every 12 hours for 6–21 days. All patients cleared their peripheral blood of blast cells but no complete remissions were achieved. Excessive toxicity to skin and mucous membranes was observed. In the doses used, this combination is too toxic for further use. Alternate treatment schedules should be explored.

[Cancer Treat Rep 66:1397–1398, 1982]

Chemotherapy for acute nonlymphocytic leukemia (ANLL) has improved significantly in recent years (1). However, the outlook for patients without matched siblings who fail primary therapy with daunorubicin, cytarabine (ara-C), and 6-thioguanine is still dismal. The introduction of 5-azacitidine (2) and AMSA (3,4) has failed to produce lasting remissions in these patients. In vitro investigations have suggested that the experimental drug pyrazofurin (PZF) may act synergistically with ara-C (5). In this study, we evaluated the activity of maximally tolerable single-agent doses of PZF with ara-C in end-stage patients with ANLL.

METHODS

Six patients with ANLL who had failed either induction or reinduction therapy with daunorubicin or doxorubicin, ara-C, and 6-thioguanine were eligible for study after giving informed consent. Patients were treated with PZF at a dose of 15 mg/m² every 12 hours. Two hours after each dose of PZF, patients received ara-C at a dose of 100 mg/m². After the first 7 days of therapy, bone marrow aspiration was performed. If the marrow was not aplastic, therapy was continued for another 3–5 days at the same doses and the marrow aspiration was repeated. This regimen was to be continued for a total of up to 21 days of therapy. If, after 21 days of treatment, the bone marrow still contained blast cells, the therapy was considered to be a failure and the patient was removed from the protocol.

Drug therapy was discontinued for toxicity only because of skin reactions severe enough to cause denudation of $\geq 10\%$ of body surface area or mucositis severe enough to cause denudation of $\geq 75\%$ of the oropharynx. No patients received diminished therapy because of hematologic toxicity.

RESULTS

All patients experienced complete clearance of blast cells from the peripheral blood and reached nadir absolute granulocyte counts of zero. Granulocyte counts remained $< 500/mm^3$ for 9–62+ days. Bone marrow examination on Day 8 in the five patients examined demonstrated marked hypocellularity of the normal hematopoietic elements but persistence of blast cells. No patient achieved an M_1 or M_2 marrow. In addition, mucositis and/or skin rash were severe enough to necessitate discontinuation of therapy before the planned 21 days in five of the six patients. In three of these patients, only 1 week of therapy or less was possible. All patients who developed severe skin or mucosal reactions, however, recovered within 3 weeks.

DISCUSSION

Major improvements have been made in the treatment of adult ANLL in recent years. Therapy with combinations including ara-C, 6-thioguanine, and

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daunorubicin now produce complete remissions in upwards of 80% of patients < 50 years old (6). However, therapy for patients who relapse with this combination rarely results in prolonged disease control.

Karaues et al (7) recently reported that treatment of refractory ANLL patients with doses of ara-C in the range of 6 g/m²/day × 6 days produces a complete response rate of 50%, with a median response duration of 5+ months. Such therapy produced prolonged marrow suppression, significant stomatitis, and some cerebellar toxicity. This study suggests that increased uptake of ara-C into leukemia cells would result in clinical responses in patients refractory to standard doses of ara-C.

Another potential method of increasing activation of ara-C would be to inhibit normal de novo cytidine synthesis. Indeed, Cadman et al (5) have demonstrated that PZF, a potent inhibitor of the enzyme orotidylate decarboxylase, produces synergistic antitumor effect against the experimental L5178Y leukemia when PZF precedes ara-C treatment. The use of PZF in combination with ara-C is particularly interesting because Vogler and Trulock (8) have demonstrated that PZF itself possesses significant antileukemia activity in man. Unfortunately, doses > 30 mg/m²/day, which were necessary to obtain this effect, produced unacceptable stomatitis and skin rashes.

Therefore, in this study we utilized 30 mg/m²/day of PZF as pretreatment for ara-C. This combination produced clearing of peripheral blast cells and marrow hypoplasia in all six end-stage patients studied. However, all patients continued to demonstrate some blast cells in their bone marrows and repopulated their peripheral blood with leukemic blast cells without reappearance of normal cells. In addition, five of the six patients experienced extremely severe mucositis and skin rashes necessitating discontinuation of therapy. These results are similar to those reported for the combination of PZF and 5-azacitidine (9).

When used in this way the combination of PZF and ara-C causes unacceptable toxic reactions without producing an obvious improvement in antileukemic effect over currently available regimens. Future studies might explore other dose schedules of these two drugs. In view of the prolonged enzyme inhibition produced by large doses of PZF (5), a single dose of PZF followed by 5–7 days of ara-C would be of particular interest.

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Non-Cross-Resistant Chemotherapy and Consolidation Radiotherapy for Small Cell Carcinoma of the Lung[1,2]

Brief Reports

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SUMMARY

Fifty-six patients with small cell carcinoma of the lung were treated with a two-cycle induction course of hexamethylmelamine, vincristine, doxorubicin, and cyclophosphamide. Patients with limited disease (LD) who responded and patients with extensive disease (ED) who had a complete response received prophylactic whole-brain radiotherapy, as well as radiotherapy to thoracic and abdominal sites of disease. Concurrently with radiotherapy, consolidation chemotherapy was given with doxorubicin, cyclophosphamide, methotrexate, and etoposide. The complete response rate was 35% for ED patients and 68% for LD patients. The median survival time for complete responders was 54 weeks for ED patients and 65 weeks for LD patients. The toxicity of the program was moderate, and the effectiveness was comparable to that of other reported combined-modality treatment programs.

[Cancer Treat Rep 66:1399–1401, 1982]

Aggressive combination chemotherapy for small cell carcinoma of the lung (SCCL) can yield complete responses, and a number of groups have employed combined-modality therapy with the goal of effecting long-term survival and cure in this disease (1–3). The treatment program described here was developed by the San Diego Oncology Group to incorporate new chemotherapy agents and scheduling techniques, together with consolidative radiotherapy, in an effort to improve treatment results in SCCL.

PATIENTS AND METHODS

Patients with histologically documented SCCL and a Karnofsky performance status of at least 20 were eligible for the study. On the basis of initial staging studies, patients were designated as having limited disease (LD) if their involvement was confined to one hemithorax, the mediastinum, and ipsilateral supraclavicular lymph nodes. All other patients were designated as having extensive disease (ED).

Induction chemotherapy (HexaVAC) was given in two 28-day cycles as follows: 100 mg/m^2 of hexamethylmelamine orally on Days 2–15, 1.5 mg/m^2 of vincristine (maximum, 2.0 mg) iv on Days 1 and 8, 500 mg/m^2 of cyclophosphamide iv on Days 1 and 8, and 25 mg/m^2 of doxorubicin iv on Days 1 and 8. After two cycles of HexaVAC, six alternating 28-day chemotherapy cycles (CMV/AMV) were administered as follows: 1.0 g/m^2 of cyclophosphamide on Day 1, 200 mg/m^2 of etoposide iv on Day 1, and 30 mg/m^2 of methotrexate iv on Day 21 (CMV), followed by 50 mg/m^2 of doxorubicin iv on Day 1, 200 mg/m^2 of etoposide iv on Day 1, and 30 mg/m^2 of methotrexate iv on Day 21 (AMV).

Radiotherapy was given to all LD patients who achieved a complete response (CR) or partial response (PR) with induction chemotherapy and to ED patients who achieved a CR during induction chemotherapy. Treatment began concurrently with the first CMV cycle. Prophylactic whole-brain radiation was given as 2000 rad in ten fractions over 2 weeks. Therapy to the chest was given in two

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TABLE 1.—Response rates and survival data by stage of disease and degree of response

Disease stage	No. of patients	% response		Median survival (wks)		
		CR	PR	CR	< CR	Overall
ED	34	35	26	54	39	42
LD	22	68	14	65	36	60
All patients	56	48	21	61	38	50

courses, each consisting of 2000 rad in ten fractions over 2 weeks. The second half of radiotherapy was given during the second CMV cycle, after a break of approximately 6 weeks. The thoracic radiotherapy treatment volume encompassed the entire pretreatment area of the primary tumor and included the mediastinal and ipsilateral hilar nodes. In addition to thoracic radiotherapy, ED patients who attained a CR while receiving induction chemotherapy were given 2000 rad of consolidative irradiation to areas of known intra-abdominal disease.

All patients entered in the study and receiving any therapy were considered to be evaluable. CR was defined as the total disappearance of all evidence of disease; however, CR was not documented by bronchoscopy in most cases. PR was defined as a > 50% reduction in the product of the two largest perpendicular diameters of measurable disease. The assessment of whether chest relapse occurred within irradiated fields was made by direct comparison of port films with serial chest X-rays. Projected median survival was calculated using the technique of Kaplan and Meier, and differences in survival were assessed using the Mantel-Haenszel method.

RESULTS

Between July 1978 and October 1980, a total of 56 patients, 45 men and 11 women, were entered in the study. The median age was 60 years (range, 43–81), and the median Karnofsky performance status was 90 for LD patients and 70 for ED patients. The median followup at the time of analysis was 85 weeks.

The response rate and survival data are presented in table 1. Overall, 69% of the patients had at least a PR and 48% of the patients had a CR. The CR rate was 68% for LD patients and 35% for ED patients. The median survival time was 42 weeks for ED patients and 60 weeks for LD patients ($P = 0.006$). Complete responders in the ED group had a median survival of 54 weeks, and those in the LD group had an estimated median survival of 65 weeks. Of the ED patients, only two are alive and free of disease (40+ and 65+ weeks). In the LD group, ten patients are alive and free of disease, with five beyond 1 year (62+, 76+, 88+, 109+, and 124+ weeks). In both groups, survival for patients achieving a CR was significantly better than for those who did not ($P < 0.01$). Of the CRs, 44% occurred during induction chemotherapy and 56% occurred during the consolidation phase of treatment. The survival of patients in the early CR and late CR groups was not significantly different.

Of the 29 patients who received protocol thoracic radiotherapy, ten (40%) had disease recurrence within the radiotherapy field. Two ED patients were given hepatic irradiation for consolidation after attaining CR. Both patients had pronounced thrombocytopenia after treatment, and both had disease recurrence in the liver. Of 37 patients receiving prophylactic whole-brain radiotherapy, three (8%) had brain recurrence documented by computerized tomographic scan.

Severe leukopenia (wbc count < 2000/mm^3) occurred in 21% of patients during HexaVAC chemotherapy and in 16% of patients during the CMV/AMV phase of therapy. All other toxic reactions were graded as severe or life-threatening in < 10% of the cases. Five deaths, all in ED patients, occurred within the first 8 weeks of therapy. Two patients died from sepsis during treatment-induced leukopenia, one patient had an apparent myocardial infarction, and two patients died with progressive tumor. Toxicity from concurrent radiotherapy and CMV/AMV chemotherapy was moderate and manageable. Two patients had transient cessation of all oral intake due to esophagitis, and one patient required hospitalization for radiation pneumonitis. Chemotherapy dose reduction or delay was required for 11% of the HexaVAC cycles, 5% of the CMV/AMV cycles given without concurrent radiotherapy, and 31% of the CMV/AMV cycles given with concurrent radiotherapy.

DISCUSSION

Hexamethylmelamine, an investigational agent with a response rate of 43% as a single agent in SCCL (4), was added to an effective and established three-drug combination regimen (CAV) to form a four-drug induction regimen (HexaVAC). This combination was well-tolerated and yielded, after only two cycles, a 36% CR rate in the LD patients and

a 12% CR rate in the ED patients. When compared to response rates achieved by other groups using CAV alone (5,6), these data are encouraging and may warrant further study of the HexaVAC combination in randomized trials. After induction chemotherapy, non-cross-resistant chemotherapy was prescribed in an attempt to increase the CR rate. Etoposide, an investigational agent with 45% activity in SCCL (7), and methotrexate were added in the CMV/AMV consolidation chemotherapy. Although dose reduction or delay was required in a significant number of chemotherapy cycles given with concurrent radiotherapy, over one half of the CRs in the present study occurred during this phase of treatment.

Thoracic radiotherapy was used to achieve further cytoreduction at bulky sites of disease. Split-course radiotherapy was used to minimize the local toxic reactions associated with standard scheduling and to permit uninterrupted chemotherapy. This study demonstrated the feasibility and patient tolerance of such scheduling. A 40% relapse rate within the irradiated volume occurred in spite of the fact that thoracic radiotherapy was given only to patients achieving significant reduction in tumor bulk with induction chemotherapy. This corroborates the observation of other investigators that thoracic radiotherapy, given in several different doses and fractionation schedules, has been unsuccessful in preventing local relapse in SCCL (8,9). Only 8% (< 20% with 95% confidence) of patients receiving 2000 rad of prophylactic whole-brain radiotherapy had brain relapse. This relapse rate is comparable to that observed in other series employing higher doses (1,2).

Long-term survival in SCCL continues to be an elusive goal, and in most series < 15% of LD patients survive > 2 years (1–3). The present treatment program was reasonably successful in inducing CR, but most of these patients eventually relapsed and died from recurrent, drug-resistant neoplasm. On balance, the results of the present study are comparable to the best reported series of combined-modality therapy for SCCL.

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Cisplatin, Cyclophosphamide, and Prednisone Therapy for Stage D Prostatic Cancer[1]

Brief Reports

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Stage D prostatic cancer has a variable natural history with a median patient survival of 1–3 years (1). The objective response to chemotherapy is difficult to document (2–4). Both cisplatin and cyclophosphamide as single agents have demonstrated some activity in the management of prostatic cancer (5,6). Prednisone was selected to act on those cancer cells that might remain hormonally sensitive.

PATIENTS AND METHODS

This phase III study was initiated in March 1977 in patients with hormonally resistant stage D prostatic cancer. Patients were given cyclophosphamide (150 mg/day) for 14 days orally, cisplatin (50 mg/m^2) iv on Day 8, and prednisone (5 mg) orally three times daily, continuously throughout the 28-day cycle. Before and after hydration, steps were taken to minimize the risk of cisplatin-induced nephrotoxicity; the dose was reduced by 50% in the presence of a reduced creatinine clearance (< 50 ml/minute), and cisplatin administration was discontinued when the clearance was < 50% of pretreatment values.

The dose of cyclophosphamide was modified if the segmented neutrophil count was < 2000/mm^3 or if the platelet count was < 100,000/mm^3. Termination of treatment occurred when objective progression of disease was evident after two full cycles of therapy.

Patient Characteristics

Twenty-two patients were entered in the study until December 1980. The median age was 64 years (range, 51–80). All had received prior hormonal therapy in the form of orchiectomy, diethylstilbestrol, and/or diethylstilbestrol diphosphate. Eleven patients had received irradiation previously. Sites of disease were bone in 22 patients and lymph nodes in five; none of the patients had visceral disease. Performance status (PS) was assessed according to the Eastern Cooperative Oncology Group criteria; one patient had PS 0; eight, PS 1; three, PS 2; five, PS 3; and five, PS 4.

RESULTS AND DISCUSSION

Responses were defined in accordance with the criteria set by the National Cancer Project. No objective or partial responses were documented in the 22 patients; ten (45%) achieved stable disease status and one had subjective improvement despite progressive disease. The overall median survival was 9 months; median survival was 10 months in patients with stable disease and 5 months in patients with disease progression. The median duration of response was 6 months. No statistically significant difference in survival was demonstrated between responders and nonresponders. Three of five patients with measurable nodes had a > 50% reduction in their size but failed to have a corresponding decrease in acid phosphatase to normal levels, required to define a partial remission. Seven patients in the objectively stable group had a > 50% reduction in serum acid phosphatase, and three had a return of alkaline phosphatase levels to normal. Subjective improvement as defined by decreased pain, improvement in performance status, and weight gain was seen in the ten objectively stable patients as well as in one patient who had disease progression by National Cancer Project criteria. The median number of cycles of therapy was four (range, 1–14). Toxicity was within acceptable limits, considering the number of patients who had received prior irradiation and who had extensive bone marrow involvement. Only three patients experienced significant neutropenia with segmented neutrophil counts < 1000/mm^3. Two of these three had coexistent thrombocytopenia of < 100,000 but > 20,000/mm^3. There was no evidence of disseminated intravascular coagulation in these patients.

Despite the age and poor general status of many patients, cisplatin was reasonably well-tolerated. Only three patients had initial reduction in cisplatin dose due to low pretreatment creatinine clearances.

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There was no significant deterioration in creatinine clearance values after discontinuation of cisplatin in the face of advancing disease. Eight patients experienced moderate to severe nausea, with only four having moderate to severe vomiting (more than four episodes within a 24-hour period). One patient developed ototoxic effects secondary to cisplatin.

In this small unselected group, our results did not show any superiority to those achieved by single-agent therapy; approximately 50% of the patients experienced improved pain control with diminished analgesic requirements and improved performance status. In contrast to other studies, the objectively stable patients (45%) did not gain a significant survival benefit. The addition of prednisone to the regimen may have interfered with analysis by improving appetite and sense of well-being. Whether the administration of prednisone altered the androgenic pathway in addition to the expected glucocorticoid suppression is not known, because hormonal parameters were not measured during the course of treatment. It became apparent that unless significant pain relief occurred after one cycle of treatment, further therapy would fail. Most treatment, with the exception of the initial course, was done on an outpatient basis.

The initial response to hormonal maneuvers in stage D prostatic cancer is usually about 80%. However, because the disease will ultimately progress and because some patients are hormonally resistant to initial treatment, there is a need to develop more effective chemotherapeutic regimens. The combination of cisplatin, cyclophosphamide, and prednisone in our group of patients provided good short-term palliation with acceptable toxicity, although it did not show any clear-cut benefit over other combinations or over single-agent therapy currently used.

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VAB-3 Combination Chemotherapy in Primary Mediastinal Germ Cell Tumors[1,2]

Brief Reports

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SUMMARY

Ten patients with primary mediastinal nonseminomatous germ cell tumors were treated with the VAB-3 protocol. All patients with measurable disease showed tumor regression with chemotherapy, but only two had sustained partial response allowing thoracotomy and resection of residual tumor after the second induction. Both patients received additional induction therapy and one also received irradiation; they have been free of disease 41+ and 40+ months from the beginning of chemotherapy. All other patients died from the disease.

[Cancer Treat Rep 66:1405–1407, 1982]

Primary germ cell tumors of the mediastinum account for 1%–4% of tumors arising in the mediastinum (1,2) and are histologically identical to those arising in the testis. Distinction between seminomatous and nonseminomatous tumors determines the prognosis and treatment. Seminoma accounts for about 30% of all primary mediastinal germ cell tumors, and surgery alone or combined with irradiation results in apparent cure in about 50% of patients (2–6). The results with nonseminomatous germ cell tumors are poor (3,7). This is a review of ten patients with primary mediastinal germ cell tumors treated with VAB-3 combination chemotherapy.

MATERIALS AND METHODS

Between September 1975 and February 1978, ten male patients with apparent primary mediastinal germ cell tumors were entered in the VAB-3 protocol at the Memorial Sloan-Kettering Cancer Center (MSKCC). Eight of these patients were white and two were black. Nine patients had nonseminomatous germ cell tumors and one had a biopsy-proven seminoma but elevated serum alpha-fetoprotein and beta-human chorionic gonadotropin, indicating the presence of nonseminomatous elements. The diagnosis was established in all patients by thoracotomy or mediastinotomy and biopsy. Histologic diagnosis was made by the Pathology Department of MSKCC. Two patients had received mediastinal irradiation and one other had received chemotherapy prior to referral to MSKCC. Nine patients had measurable disease that was bulky in eight and nonbulky (< 5 cm in diameter) in one. One patient had complete resection of all gross disease and no measurable tumor at the beginning of chemotherapy.

To assess extent of disease prior to chemotherapy, each patient had a workup that included physical examination, chest X-ray, bone scan, liver and spleen scans if liver function was abnormal, and neurologic computerized tomographic scan, when indicated. A number of patients had pedal lymphangiogram and iv pyelogram.

The VAB-3 regimen consisted of two hospital inductions of 8 days each, 5–6 months apart, and outpatient treatments every 3 weeks (table 1). Prior to each induction, each patient had a pulmonary function test and a 24-hour creatinine clearance.

Responses were defined as complete and partial. Complete response was defined as the elimination of all evidence of disease for a minimum of 3 months. All other patients were partial responders. There was no dose modification for chemotherapy. If the leukocyte count was < 3800/mm^3 or the platelet count < 150,000/mm^3, chemotherapy was delayed until recovery.

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TABLE 1.—VAB-3 schedule

Day or treatment No.	Drug	Dose and route
	Induction	
Day 1	Cyclophosphamide	600 mg/m² iv
	Vinblastine	4 mg/m² iv
	Dactinomycin	1 mg/m² iv
	Bleomycin	30 mg iv
Days 1–7	Bleomycin	20 mg/m²/day by continuous infusion for 7 days
Day 8	Cisplatin	120 mg/m² iv with mannitol-induced diuresis
	Consolidation 3 wks later: treatment every 3 wks × 6	
Treatment 1 and 4	Vinblastine	4 mg/m² iv
	Doxorubicin	30 mg/m² iv
	Chlorambucil	4 mg/m² orally daily for 14 days
Treatment 2 and 5	Vinblastine	4 mg/m² iv
	Dactinomycin	1 mg/m² iv
	Chlorambucil	4 mg/m² orally daily for 14 days
Treatment 3 and 6	Vinblastine	4 mg/m² iv
	Cisplatin	50 mg/m² iv
	Chlorambucil	4 mg/m² orally daily for 14 days
	Reinduction 3 wks later: same as induction	
	Maintenance 3 wks later: treatment every 3 wks for 18 mos	
	Vinblastine	4 mg/m² iv
	Dactinomycin	1 mg/m² iv
	Chlorambucil	4 mg/m² orally daily for 14 days

RESULTS

Ten patients received the initial VAB-3 induction therapy and all nine with measurable disease showed regression of tumor. In seven patients (including one without measurable disease at the start of chemotherapy), however, the disease progressed before the scheduled second induction 5–6 months later. Three patients with sustained partial response to the first induction received a second: one subsequently progressed and two had thoracotomy approximately 8 weeks after the last induction. In one patient microscopic foci of embryonal carcinoma were resected from the pericardium and he received an additional two inductions; he remains free of disease 40+ months from the beginning of chemotherapy. The other patient who had bone metastases at the beginning of treatment had 99% resection of the primary tumor, irradiation to the humerus, two further inductions, and irradiation to the mediastinum; he is free of disease 41+ months from the beginning of chemotherapy and 36+ months from the last surgery.

One patient had a temporary increase in serum creatinine (> 2 mg/100 ml) after a second induction with cisplatin. One patient was treated with broad-spectrum antibiotics when he developed fever during myelosuppression.

DISCUSSION

Effective chemotherapy appears essential for the control of primary mediastinal nonseminomatous germ cell tumors but the rarity of this tumor and the recent evolution of effective chemotherapy have limited accumulation of relevant experience. Current policy is to treat patients with primary mediastinal nonseminomatous germ cell tumors with chemotherapy regimens identical to those used for histologically similar germ cell tumors of the testis. During the past decade, combination chemotherapy, variously incorporating cisplatin, bleomycin, vinblastine, and other agents, has improved results in the treatment of disseminated testis cancer. Currently, the potential for cure of patients with advanced germ cell tumors of the testis is approximately 80% (8).

The results presented here for the treatment of primary mediastinal germ cell tumors are worse than those reported for treatment of testis cancer with the VAB-3 regimen, probably because patients with primary mediastinal germ cell tumors have a greater bulk of disease at the beginning of chemotherapy (9). It is our impression that patients with primary mediastinal germ cell tumors have a response to chemotherapy alone which is similar to

that of patients with equally bulky germ cell tumors of the testis.

A sustained response to chemotherapy was crucial for complete remission, but both patients achieving such a response required additional focal treatment with surgery and/or irradiation to achieve complete remission. All patients who showed tumor progression in spite of chemotherapy died.

Despite recent optimism in the treatment of patients with nonseminomatous primary mediastinal germ cell tumors, cures are still the exception rather than the rule. From experience with primary germ cell testis tumors supplemented by this small experience with mediastinal germ cell tumors, one may tentatively conclude that patients with primary mediastinal nonseminomatous germ cell tumors should be treated initially with chemotherapy employing successive inductions at shorter intervals (ie, VAB-6 protocol) (8). This should be followed by an attempt at surgical resection of residual disease. When the resected tissue shows malignant elements, additional inductions are recommended.

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Vindesine (VDS) Monochemotherapy for Non-Small Cell Lung Cancer: A Report of 45 Cases[1,2]

Brief Reports

Susan L. Luedke,* Dan W. Luedke, Paul Petruska, G. O. Broun, Garry Reed, and John Leavitt[3,4]

SUMMARY

Fifty-three patients with non-small cell lung cancer were treated with vindesine. Of the 45 evaluable patients, 11 (24%) had a partial response. Responses were all evident within 6 weeks. Median duration of response was 8 weeks from documentation. Median survival was not improved significantly among responders ($P < 0.10$, two-tailed test of significance). The most frequent toxic effect was leukopenia. The most troublesome toxic effect was peripheral neuropathy, with patients > 60 years old experiencing this more frequently.

[Cancer Treat Rep 66:1409–1411, 1982]

In 1979, Gralla et al (1) reported the results of vindesine (VDS) treatment of metastatic non-small cell lung cancer (NSCLC). Ten of 46 evaluable patients had a partial response (PR). In light of this promising report, we initiated trials of VDS in patients with advanced epidermoid carcinoma and adenocarcinoma of the lung.

MATERIALS AND METHODS

Patients were eligible for treatment if they had pathologically proven NSCLC that was incurable and measurable; had normal renal, hepatic, and bone marrow function (unless tumor-related abnormalities were present); had an expected survival of at least 6 weeks; and had given written informed consent.

VDS was administered at a dose of 3 mg/m² (increased to 4 mg/m²) by iv bolus injection once weekly for 10 weeks, followed by 3 mg/m² biweekly. Dose attenuation was made on the basis of hematologic and neurologic toxicity as previously described by Gralla et al (1). Patients were considered evaluable for response if they were in the study 6 weeks (or less with documented disease progression). Response to therapy was defined as follows: *PR*—a $\geq 50\%$ reduction in tumor size; *minor response*—a $< 50\%$ but $\geq 25\%$ reduction in tumor size; *stable disease*—neither response nor disease progression for at least 6 weeks; and *progressive disease*—a $\geq 25\%$ increase in tumor size or appearance of new disease outside the central nervous system. Duration of response was measured from the time of documentation, and response had to be present for a minimum of 1 month.

RESULTS AND DISCUSSION

Fifty-three patients were treated with VDS, with 85% fully evaluable. Twenty-eight patients with epidermoid carcinoma were part of a protocol comparing VDS with cyclophosphamide and doxorubicin; they will also be reported on elsewhere. Of the eight inevaluable patients, seven died within the first 3 weeks; death was unrelated to therapy and there was no measurable change in disease. The remaining patient had an erroneous pathology diagnosis.

Patient characteristics in relation to response are shown in table 1. Weight loss, extensive disease [by Veterans Administration Lung Group criteria (2)], and prior radiation therapy were associated with poor response to VDS. Eleven (24%) of the patients had a PR with a median duration of 8 weeks. An additional four patients had a minor response. This response rate (24%) is remarkably similar to the 21% reported by Furnas et al (3) in their compilation of 146 patients with NSCLC treated with VDS monotherapy. Response was evident within the first 6 weeks of therapy. Sites of response were the pri-

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[4]We thank Jean Schlueter, Linda Hackney, and Angelia Estes for their assistance.

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mary lesion and lymph nodes (bone metastases were not considered measurable). Symptomatic brain metastases developed while patients were responding peripherally. Otherwise, relapse occurred principally in sites of measurable disease. Median survival was 14 weeks (fig 1) with no significant difference in survival between patients responding and those with stable or progressive disease ($P < 0.10$). This must be interpreted with caution as the treated patient population was heterogeneous in prognostic characteristics.

Toxic effects due to VDS were principally neutropenia and neurotoxicity. Neutropenia was the most frequent dose-limiting toxic reaction (table 2).

TABLE 1.—Patient characteristics and response to VDS chemotherapy

Characteristic	Total No. of patients (%)	No. of patients (%) with— PR	PR + MR*
Karnofsky performance status			
80–100	36 (80)	10 (28)	14 (39)
≥ 60, < 80	5 (11)	0	0
≤ 50	4 (9)	1	1
Epidermoid carcinoma	35 (78)	9 (26)	13 (37)
Adenocarcinoma	10 (22)	2 (20)	2 (20)
Extensive disease	34 (76)	3 (9)	6 (18)
Weight loss > 10%	19 (44)	2 (10)	4 (22)
Sex			
Male	39	10	14
Female	6	1	1
Prior radiotherapy	17 (38)	2 (12)	2 (12)
Prior chemotherapy	6 (13)	1 (17)	2 (33)

*MR = minor response.

TABLE 2.—VDS hematologic toxicity

Toxicity	No. of patients (%)
Wbc count (cells/mm³)	
≥ 4000	11 (24)
< 4000, ≥ 2500	17 (38)
< 2500	17 (38)
Platelet count (cells/mm³)	
> 450,000	14 (31)
< 150,000, ≥ 100,000	12 (27)
< 100,000, ≥ 50,000	1
< 50,000	1

TABLE 3.—VDS neurotoxicity

Characteristic	Total No. of patients	No. of patients with neurotoxicity (%)	Significance (chi-square)
Age in yrs			
> 60	26	10 (38)	$P < 0.05$
≤ 60	19	13 (68)	
Total VDS (mg/m²)			
> 18	24	15 (63)	$P < 0.10$
≤ 18	21	8 (38)	
Evaluable	45	23 (51)	

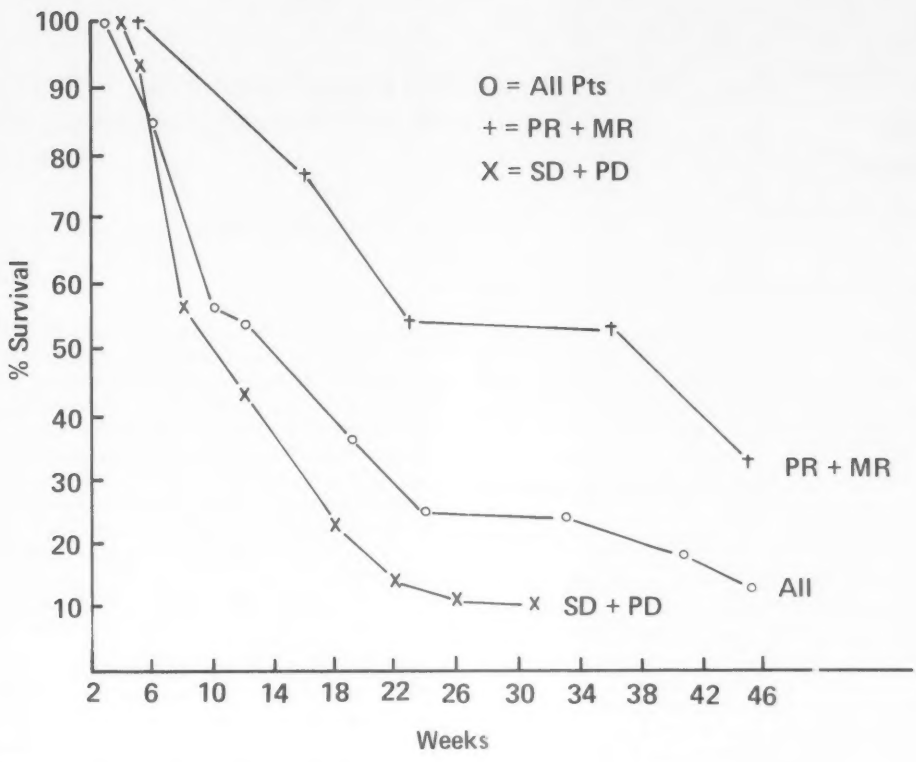

FIGURE 1.—Survival of all patients with NSCLC treated with VDS compared to those with PR or minor response (MR) and those with stable disease (SD) or progressive disease (PD).

No life-threatening infection or bleeding episode was found as a result of bone marrow suppression. Peripheral neuropathy was the most troublesome toxic effect (table 3), with patients > 60 years of age experiencing this more frequently ($P < 0.05$). Although dose-dependent neuropathy with VDS has been reported elsewhere, this age-dependent neuropathy has not been described in other VDS clinical studies nor in two recent reviews of vincristine neurotoxicity (4–6).

We conclude that VDS is an active drug in NSCLC. Dose increase above 3 mg/m^2 does not appear to be warranted.

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Treatment of Advanced Plasma Cell Myeloma With Weekly Cyclophosphamide and Alternate-Day Prednisone[1]

Brief Reports

Lorne J. Brandes* and Lyonel G. Israels[2,3]

SUMMARY

Three of five patients with refractory myeloma were successfully palliated using a simple and relatively nontoxic regimen consisting of weekly cyclophosphamide in combination with alternate-day oral prednisone. Control of advanced disease in some patients may be associated with more frequent scheduling of known active drugs, even though these drugs, either alone or in combination, may not be effective when given at standard intervals of 4–6 weeks.

[Cancer Treat Rep 66:1413–1415, 1982]

Approximately 50% of patients with multiple myeloma achieve remission when treated with either melphalan or cyclophosphamide as single agents in association with prednisone (1). With the possible exception of a five-drug combination (2), the remission rate and survival in this disease have improved only marginally with the use of more aggressive combination chemotherapy, either in alternating or sequential schedules (3–5).

Regardless of the type of induction therapy, remission is achieved in most cases with < 2-log cell kill (6). A plateau phase follows where, despite continuing cyclic therapy, a further reduction in M protein does not occur (7). Eventually, an accelerated phase of the disease, characterized by an increase in labeling index, occurs (8). Treatment of patients in this malignant phase has tended to be more aggressive, but despite the use of multiple-drug regimens, overall survival has been poor and drug toxicity has been high (9).

We report the palliative treatment of five patients with advanced myeloma, using a simple and relatively nontoxic regimen consisting of weekly cyclophosphamide (150–300 mg/m^2 iv) in combination with alternate-day oral prednisone (50–100 mg).

MATERIALS AND METHODS

Patients

Five patients with advanced myeloma who were either initially or subsequently refractory to melphalan and prednisone and then to multiple-drug regimens were treated with weekly cyclophosphamide and alternate-day prednisone.

The treatment regimen consisted of alternate-day oral prednisone (50–100 mg) and weekly low-dose iv cyclophosphamide (150–300 mg/m^2; maximum weekly dose, 500 mg). Because a 5-month switch to oral weekly cyclophosphamide from the iv route in patient 1 resulted in disease progression which was corrected within 2 months of reinstitution of the iv schedule, all subsequent doses of cyclophosphamide in this patient and in the subsequent patients were given iv.

Patients were considered evaluable after having completed at least eight weekly injections of cyclophosphamide.

Response to therapy was defined as a sustained decrease in M protein (< 50% of the pretreatment value), a significant improvement in anemia or pancytopenia, correction of hypercalcemia where this condition existed, and stabilization of lytic bone lesions.

RESULTS

The results of therapy are shown in table 1. Three of five patients (Nos. 1–3), each with a serious complication of this disease, responded well to therapy. Patients 4 and 5 failed to respond and died within 2 months of starting weekly cyclophosphamide and

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TABLE 1.—Patient characteristics and response to therapy*

Patient No.	Sex/age (yrs)	Type	Previous treatment	M protein level (serum = g/dl; urine = g/24 hrs)		Hemoglobin (g/dl)		Complication(s) at time of initiation of CP	Result of CP therapy	Duration of response†
				Before CP	After CP	Before CP	After CP			
1	M/52	IgG κ	MP (2½ yrs); BAP (13 mos); C × 1 course (1 g over 4 days; failed to respond)	1.6 g/dl	0.6 mg/dl	7	12	Leukemic phase (plasma cells = 9000/μl); pancytopenia	Resolution of pancytopenia and plasmacytosis	24 mos‡
2	F/52	IgA λ	MP (8 yrs); VBAP q4wks × 7 courses + radiotherapy (failed to respond)	3.0 g/dl	0.8 g/dl	9	12	Hypercalcemia	Resolution of hypercalcemia	14+ mos
3	F/68	IgG κ	MP × 3 courses; VMCP q4wks × 3 courses (failed to respond)	5.6 g/24 hrs	0.7 g/24 hrs	7	12.5	Myeloma kidney; creatinine clearance (< 5 ml/min); pancytopenia	Increase in creatinine clearance (39 ml/min); normalization of Hgb and platelets; wbc remains low (2.5×10^3 cells/mm^3)	12+ mos
4	F/72	Light chain disease	MP (18 mos); VBAP × 3 courses; VCMP × 3 courses (failed to respond)	2.0 g/24 hrs	Not quantitated	7	7	Hypercalcemia	Failed to respond (10 courses)	—
5	M/66	IgG λ	MP × 6 courses VBAP × 2 courses; VMCP × 3 courses; (failed to respond)	3.5 g/dl	5.0 g/dl	8	7	Pancytopenia	Failed to respond (8 courses); hypercalcemia	—

*M = melphalan; P = prednisone; V = vincristine; B = BCNU (carmustine); A = doxorubicin; CP = weekly cyclophosphamide and alternate-day prednisone.

†Duration of response is calculated from date CP started.

‡Patient died from septicemia while still responding to CP.

alternate-day prednisone. The responding patients had a prolonged survival, with patients 2 and 3 still receiving therapy at 14+ and 12+ months, respectively. After 24 months of receiving treatment, and while still responding, patient 1 suddenly developed an overwhelming septicemia and died; *Listeria monocytogenes* was cultured from his blood and spinal fluid.

Toxic effects associated with this therapy were limited to one episode of herpes zoster in patient 3 which resolved within 2 weeks. Nausea and vomiting were minimal to nonexistent in all patients; alopecia was not seen. Patients 1 and 3 had disease-associated pancytopenia that subsequently improved with therapy.

DISCUSSION

Myeloma resistant to melphalan has a poor prognosis. Recent reports of response to aggressive multidrug therapy in advanced myeloma have been disappointing. The Southeastern Cancer Study Group reported a 13% response rate in patients treated at 4-week intervals with a combination of BCNU, cyclophosphamide, and prednisone (BCP) after initial failure of melphalan and prednisone therapy (10). In a similar trial, Kyle et al (11) reported an objective response rate of 17% in 42 melphalan-resistant patients treated with BCP every 6 weeks and of only 7% in 47 similar patients treated with cyclophosphamide and prednisone every 6 weeks.

A five-drug protocol (M2) consisting of BCNU, cyclophosphamide, melphalan, vincristine, and prednisone given in 5-week cycles has produced good responses in 13 of 26 patients who were previous treatment failures (2). However, only one of five patients who stopped therapy and entered the aggressive phase of the disease responded to reinstitution of this regimen. In addition, toxicity associated with this treatment included nausea, vomiting, leukopenia, infection, and hemorrhage (2).

Houwen et al (12) have reported the successful palliation of six of 11 patients with advanced myeloma who were treated with a combination of prednisone and vindesine. In this study the vindesine was given weekly for three injections followed by a 3-week rest interval and then repeated.

We suggest that success in controlling advanced disease in some patients may be achieved by the more frequent scheduling of known active drugs, even though the same drugs, either alone or in combination, may not be effective when given at standard monthly intervals. This may also account for the discrepancy between an earlier study of Bergsagel et al (13) demonstrating that cyclophosphamide benefited six of 19 patients with melphalan-resistant myeloma when given at a dose of 1 g/m^2 every 3 weeks, and that of the same investigator in the National Cancer Institute of Canada study which showed only a 5% response rate in melphalan-resistant patients when cyclophosphamide was given in a higher dose at 4-week intervals (4). Similarly, the Southwest Oncology Group has noted the highest response rates in schedules with the shortest treatment intervals (3). The response rate of 55% achieved by Houwen et al (12) to vindesine at weekly intervals is consistent with this concept.

The successful palliation of three of five patients with advanced myeloma using a simple regimen is of clinical importance. Moreover, the three responding patients had serious complications of their disease and in each case control was achieved with little or no associated drug toxicity. The National Cancer Institute of Canada is currently evaluating this approach in the treatment of patients with advanced myeloma who are refractory to melphalan and prednisone.

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Phase II Study of Cisplatin, Maytansine, and Chlorozotocin in Small Cell Lung Carcinoma (EST 2578)[1,2]

Brief Reports

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SUMMARY

Seventy-three patients with small cell lung carcinoma refractory to standard chemotherapy were entered in this phase II randomized study of cisplatin, maytansine, and chlorozotocin. Of the 58 evaluable patients, only one partial response was observed among 21 patients given cisplatin, and no responses were seen among 19 given maytansine or 18 given chlorozotocin. One patient treated with chlorozotocin and two treated with cisplatin experienced life-threatening thrombocytopenia. One third of the maytansine-treated patients experienced moderate or severe neurologic toxicity. The overall median survival was 9.7 weeks. Chlorozotocin treatment was associated with inferior survival (7.7 weeks).

[Cancer Treat Rep 66:1417–1419, 1982]

In this phase II study of small cell lung carcinoma refractory to standard chemotherapy, patients were randomized to receive one of three chemotherapeutic agents that had not previously received adequate clinical trial: cisplatin, the ansa macrolide antibiotic maytansine, and the nitrosourea chlorozotocin.

PATIENTS AND METHODS

Patients were stratified by Eastern Cooperative Oncology Group (ECOG) performance status (PS) (ambulatory, PS 0 or 1 versus nonambulatory, PS 2) as well as by disease extent (limited versus extensive).

To be eligible for entry in this study, each patient had to be refractory to standard chemotherapy and had to have measurable or evaluable disease, an ECOG PS of 0–2, a wbc count $\geq 4000/mm^3$, a platelet count $\geq 100{,}000/mm^3$, a BUN ≤ 25 mg/100 ml, a creatinine < 1.5 mg/100 ml, and a bilirubin ≤ 2.0 mg/100 ml. All patients gave informed written consent prior to participation in this study.

Patients randomized to cisplatin received 75 mg/m^2 iv every 21 days after adequate hydration and diuresis. Maytansine was given at 1.5 mg/m^2 iv every 21 days and chlorozotocin was given at 120 mg/m^2 iv every 42 days. Each drug was given until there was clinical evidence of progressive disease, after which patients were not eligible for treatment with the other study drugs.

Objective tumor response was classified according to the standard ECOG response criteria, and treatment complications were reported according to ECOG standard criteria by degree (none, mild,

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[2]This study was conducted by the Eastern Cooperative Oncology Group (*P. P. Carbone*, chairman, CA-21115) and was supported by Public Health Service grants from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The following members participated in this study: American Oncologic Hospital, Fox Chase Cancer Center, Philadelphia, PA (*R. H. Creech*, CA-18281); Sidney Farber Cancer Institute, Boston, MA (*K. Stanley*, CA-23318); Albert Einstein College of Medicine, Bronx, NY (*S. E. Vogl*, CA-14958); The Johns Hopkins Oncology Center, Baltimore, MD (*D. S. Ettinger*, CA-16116); Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL (*P. D. Bonomi*, CA-10948); and University of Rochester Cancer Center, NY (*O. Salazar*, CA-11083). Other participating institutions include: Albany Medical College, NY (CA-06594); Baystate Medical Center, Springfield, MA (CA-20182); Case Western Reserve University, Cleveland, OH (CA-14548); Chicago Medical School, IL (CA-14144); Harbor General Hospital, Torrance, CA (CA-21091); Jefferson Medical College, Philadelphia, PA (CA-14215); University of Manitoba, Winnipeg, Canada; New York University, New York (CA-16395); Northwestern University Medical Center, Chicago, IL (CA-17145); Hospital of the University of Pennsylvania, Philadelphia (CA-15488); Roswell Park Memorial Institute, Buffalo, NY (CA-12296); University of Pretoria, South Africa (CA-21692); State University of New York-Stony Brook (Veterans Administration Hospital), Northport, NY (CA-20161); Tufts University, Walpole, MA (CA-07190); Natalie Warren Bryant Cancer Center, Tulsa, OK; Medical College of Virginia, Richmond (CA-10572); Wisconsin Clinical Cancer Center, Madison (CA-21076); and Yale School of Medicine, New Haven, CT (CA-15485).

[3]We thank Joann Keesey for excellent technical assistance.

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moderate, severe, life-threatening, or lethal), type, and cause (1).

To avoid possible influences of imbalances caused by various prognostic factors, the analysis of the survival data was conducted using the proportional hazards model proposed by Cox (2–4).

RESULTS

Seventy-three patients were entered in this study between September 1978 and October 1979. Twenty-one of 25 patients admitted to the cisplatin arm, 19 of 25 patients admitted to the maytansine arm, and 18 of 23 patients admitted to the chlorozotocin arm were analyzed. Fifteen patients were considered unevaluable (seven had unacceptable laboratory values, the condition of five deteriorated prior to initiation of therapy, two had a PS > 2, and one refused treatment).

Of the 58 evaluable patients, all had previously received combination chemotherapy regimens: 21 had received one prior regimen, 25 had received two regimens, nine had received three regimens, two had received four regimens, and one had received seven regimens. The number of patients exposed to individual chemotherapeutic agents was as follows: 54, cyclophosphamide; 48, doxorubicin; 43, vincristine; 42, a nitrosourea; 34, methotrexate; 19, procarbazine; 15, etoposide; three, dacarbazine; two, hexamethylmelamine; one, 5-FU; and one, bleomycin. Sixty-five percent of these patients had previously had chemotherapy-induced responses.

Among the three treatment arms, there were no significant imbalances in patient characteristics including age, sex, extent of disease, initial PS, prior weight loss, and prior radiotherapy. Overall, 40% of the patients were ⩾ 60 years old, 67% were male, 86% had disease extending beyond the hemithorax, 33% had a weight loss ⩾ 5% in the previous 6 months, 72% had prior radiotherapy, and 64% and 28% had a PS of 1 or 2, respectively.

There was only one partial response among the 58 evaluable patients. In this patient, one of 21 patients (5%) given cisplatin, a 1 × 1-cm skin metastasis underwent a complete regression by Day 25, while a rib metastasis remained stable for 53 days. Some degree of toxic reaction was observed in 100% of cisplatin-treated patients, in 79% of maytansine-treated patients, and in 72% of chlorozotocin-treated patients. Two patients receiving cisplatin had life-threatening thrombocytopenia (platelet count nadirs of 19,000 and 9500/mm^3, respectively). One patient receiving chlorozotocin had life-threatening gastrointestinal bleeding due to a platelet count of 20,000/mm^3. The severe toxic reactions associated with cisplatin included leukopenia (one patient), stomatitis (one), vomiting (two), and ototoxicity (one). There was no renal toxicity. Maytansine caused anemia requiring transfusions in one patient and severe neurologic toxic effects including myalgias, paresthesias, disorientation, headache, jaw pain, and weakness in four patients. One patient receiving chlorozotocin experienced severe stomatitis.

The 58 analyzed patients have died; they had an overall median survival of 9.7 weeks. The median survival times in weeks for the three regimens were 9.8 for cisplatin, 10.7 for maytansine, and 7.7 for chlorozotocin. Cisplatin-treated patients ($P = 0.005$) and maytansine-treated patients ($P = 0.02$) had longer survival times than chlorozotocin-treated patients. There was no significant difference in survival between the cisplatin- and maytansine-treated patients. Ambulatory patients (PS 0 or 1) had longer median survival times (10–12 weeks) than nonambulatory patients (PS 2; 5 weeks) ($P < 0.001$). The extent of disease and prior weight loss were not statistically significant prognostic indicators after adjusting for initial PS.

DISCUSSION

Although DeJager et al (5) reported four partial responses among 13 patients with small cell lung carcinoma treated with high-dose cisplatin (120 mg/m^2 every 3 weeks), cisplatin, maytansine, and chlorozotocin were not found to have significant antineoplastic activity in this phase II trial of patients with extensively previously treated small cell lung carcinoma refractory to chemotherapy. If an active agent is defined as one having a response rate of 20%, it can be calculated that we are 94% confident that the true response rate for this class of patients treated with cisplatin is < 20%. For maytansine and chlorozotocin, the similar confidence levels are 99% and 98%, respectively. The most severe toxic effect of cisplatin and chlorozotocin was thrombocytopenia and for maytansine was neurologic. The unexpected thrombocytopenia noted with cisplatin most likely reflects chronic marrow suppression by previous extensive radiotherapy and chemotherapy in patients entered in this trial. The short survival times reflect the multiple previous treatments of these patients and the natural history of patients with progressive small cell lung carcinoma.

Because of the relatively short survival times of chemotherapy-refractory patients with small cell lung cancer, future phase II studies should be conducted only in ambulatory patients (ECOG PS 0 or 1) to allow adequate time for response evaluation.

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Combination Chemotherapy Used Prior to Radiation Therapy for Locally Advanced Squamous Cell Carcinoma of the Head and Neck[1]

Brief Reports

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SUMMARY

Thirty-three patients with locally advanced squamous cell carcinoma of the head and neck were scheduled to receive two courses of chemotherapy prior to radical radiotherapy. Chemotherapy consisted of moderate-dose methotrexate with leucovorin rescue, bleomycin by infusion, and cisplatin. Loss of body weight and the duration of membrane formation at a specified region of the oral cavity during radiation therapy were used as indices of radiation toxicity: there was no excessive loss of body weight or mucosal reaction in patients who received combined treatment compared to patients in a nonrandomized control group who received radiotherapy alone. Twenty patients (60%) had a ⩾ 50% decrease of measurable disease prior to starting irradiation, but only eight patients (24%) are alive and disease-free at a median followup of 16 months. Aggressive chemotherapy does not prevent delivery of subsequent full-dose radiotherapy for squamous cell carcinoma of the head and neck, but this study does not suggest that chemotherapy has a great beneficial effect on long-term survival.

[Cancer Treat Rep 66:1421–1424, 1982]

Patients with locally advanced squamous cell carcinoma (SCC) of the head and neck have a high rate of local failure after irradiation and/or surgery, and there is current interest in combining chemotherapy with these modalities. When chemotherapy has been given concurrently with irradiation, toxicity has usually been high, often leading to reduced dose or protracted delivery of radiation (1–3). Chemotherapy given prior to radiotherapy seems to be better tolerated (3–5), although the effect of combined modality treatment on mucosal toxicity has not been quantitated.

In the current study we have treated patients with locally advanced, node-positive SCC with two courses of aggressive chemotherapy followed by full-dose radiotherapy. Surgery was reserved for failure of initial therapy. End points of the study were toxicity from chemotherapy, weight loss and duration of mucosal reaction during radiation therapy in patients who received combined treatment compared with a nonrandomized group of patients receiving irradiation alone, response to chemotherapy at the time of starting irradiation, and disease-free survival.

STUDY DESIGN

Patients were eligible for this study if they had locally advanced, node-positive SCC of the head and neck (table 1) and were considered suitable for radiation treatment with curative intent. All patients were ambulatory (Eastern Cooperative Oncology Group performance status 0 or 1). They were assessed with physical examination including bidimensional measurements of lymph nodes, cbc, liver function tests, serum creatinine, chest X-ray, and tomograms of local disease. Patients with serum creatinine > 1.0 mg/100 ml had measurement of creatinine clearance and were excluded if this was < 60 ml/minute. Radiation dosage and fields were planned *before* institution of chemotherapy.

Eligible patients received overnight hydration (plus bicarbonate) before receiving the following iv chemotherapy: *Day 1*—methotrexate, 100 mg/m² every 3 hours for three doses and bleomycin, 20 units/m²

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by iv push; *Days 1–4*—bleomycin, 20 units/m²/day by continuous infusion; *Day 2*—leucovorin, 15 mg/m² every 6 hours for four doses, to start 24 hours after the first dose of methotrexate; and *Day 5*—cisplatin, 60 mg/m². Serum creatinine was measured daily; leucovorin administration was prolonged and cisplatin was omitted if serum creatinine increased significantly after methotrexate. Patients were seen weekly, and serum creatinine measurement and cbc were performed at this time. Patients were reassessed at 3 weeks: if there was no disease progression or severe toxic effect, they received one further identical course of chemotherapy beginning on Day 22 and commenced radiation treatment 2–3 weeks later. Patients with disease progression or toxic effects on Day 22 had no further chemotherapy and started radiation therapy as soon as practical. Patients were reassessed, with physical examination and X-rays defining disease, prior to the start of radiation therapy.

Radiation dose and fields depended on site of disease but were those usually prescribed for patients not receiving chemotherapy. Most patients were scheduled to receive radiation to sites of disease involvement as daily fractions ranging from 5000 rads/20 fractions/4 weeks to 6000 rads/30 fractions/6 weeks. Clinically normal lower cervical nodes received 3500 rads/15 fractions/3 weeks.

Patients were seen twice weekly during and immediately after radiation treatment and were weighed and questioned about oral intake and nausea and vomiting or other symptoms. The presence or absence of membrane formation on the buccal mucosa just anterior to the retromolar trigone was recorded (6). Patients were seen at least every 2 months following radiation therapy, and patients with residual or recurrent disease were assessed for surgery.

RESULTS

Toxic Effects of Chemotherapy

Twenty-four of the 33 patients completed two courses of chemotherapy; one patient received only one course because of disease progression, and eight had only one course because of toxicity. The most common reason for withholding the second course was a rise in serum creatinine: this occurred in seven patients, never exceeded 2 mg/100 ml, and reverted to normal in all patients. Hematologic toxicity was mild, and only three patients had polymorphonuclear leukocyte counts < 1000/mm³ or platelet counts < 50,000/mm³. There were no episodes of drug-related infection, although one patient required plate-

TABLE 1.—Distribution of patients by disease site, UICC stage (1974), and response to treatment*

Site	No. of patients	UICC stage				Response		Current Status		
		T_{1-2}, N_1	T_{1-2}, N_{2-3}	T_{3-4}, N_1	T_{3-4}, N_{2-3}	To chemotherapy	After irradiation	ADF	AWD	D
Nasopharynx	7	1	4	1	1	2 CR, 4 PR	5 CR, 2 PR	4	3	0
Oropharynx and oral cavity	12	1	1	2	8	7 PR	3 CR, 7 PR	2	6	4
Hypopharynx	7	2	2	1	2	3 PR	2 CR, 2 PR	1	1	5
Larynx	7	0	1	1	5	1 CR, 3 PR	2 CR, 3 PR	1	1	5

*UICC = International Union Against Cancer, ADF = alive and disease-free, AWD = alive with disease, and D = dead.

let transfusion. Most patients had nausea or vomiting after cisplatin. There was no mucositis, ototoxicity, or lung toxicity, although two patients had skin reactions to bleomycin, and one developed pericardial pain without effusion or other sequelae. During bleomycin infusion, many patients developed fever that was controlled with steroids. Myocardial infarction occurred in two patients during or shortly after drug treatment, and one of them had a cardiac arrest from which he was successfully resuscitated. Neither patient had a history of prior heart disease or hypertension.

Toxic Effects of Radiotherapy

Twenty-three patients completed radiation therapy as originally planned including two who had split-course therapy. Of the remaining ten patients, six had interruptions of 1–3 weeks because of toxic effects, one was treated with radical radiotherapy elsewhere, and one failed to return for radiation treatment until 3 months after chemotherapy. Only two patients did not receive radical-dose radiotherapy.

In 19 patients, the retromolar trigone region received the full radiation dosage. Membrane formation on the mucosa just anterior to the trigone occurred in 14 of these patients, and the median duration of membrane formation in all 19 patients was 24 days (range, 0–42). Duration of membrane reaction has been recorded (6) for a previously treated group of 13 patients with SCC of the oral cavity who received 5000 rads/20 fractions/4 weeks without chemotherapy, and the median duration of membrane formation in that series was 21 days (range, 0–24+). Our patients lost 10.0% ± 1.1% of their body weight (mean ± SEM) after combined treatment, of which 3.1% ± 0.6% occurred after chemotherapy and before the start of irradiation. The historical control group receiving radiation therapy alone lost a mean 6.2% of body weight (6).

Response to Chemotherapy

Twenty (60%) of 33 patients had objective response to chemotherapy when assessed just prior to starting treatment with radiation (table 1); three had complete response (CR) and 17 had partial response (PR). The criterion for PR was a 50% decrease in the product of two diameters of all measurable lesions (usually neck nodes), with radiologic or other evidence of regression in the primary site. CR required disappearance of all evident disease on physical and radiologic examination. Response rate was higher among the seven patients with nasopharyngeal cancer (two with CR, four with PR) than among patients with cancer at other sites.

Response to Combined Treatment

Disease status was recorded for 30 patients at completion of radiotherapy: using the same criteria for response as defined for chemotherapy, there were 12 patients with CR, 14 with PR, and four nonresponders. No patient had disease progression during radiotherapy, and 14 patients had objective regression of disease compared with their status after chemotherapy.

At a mean followup of 16 months (range, 9–20) after starting chemotherapy, only eight patients (24%) are alive and clinically free of disease (table 1). Seven of these patients originally responded to chemotherapy (one had CR and six had PR), and one has had surgery for residual disease after irradiation. Fourteen patients have died, 13 with their original disease and one with a second primary in the esophagus. Of the remaining 11 patients, nine are alive with disease, and two are lost to followup but were known to have disease when last seen.

DISCUSSION

This study was designed to examine whether aggressive chemotherapy given prior to radical radiotherapy for locally advanced SCC of the head and neck would influence radiation toxicity and to seek evidence for improvement in response and survival that might justify a larger study. Most of our patients had only minor and reversible toxic effects from chemotherapy and no evidence of increased toxicity from radiation therapy; this contrasts with reports of increased toxicity from concurrent administration of radiation and drugs (1–3).

Our initial 60% objective response rate to chemotherapy is consistent with that found in other series in which patients with SCC received chemotherapy prior to irradiation (3–5). Despite a high initial response rate to chemotherapy, the survival of patients in this trial remains poor, and only 24% of them remain disease-free at a median followup of 16 months. Clinically impressive objective responses to chemotherapy may represent only one decade of cell kill, and further evidence for a rather limited cell kill comes from our unpublished observations of a few patients with metastatic disease who had only short responses to the same chemotherapy when no subsequent irradiation was given. This rather small cell kill (on a logarithmic scale) added to the larger effect of irradiation may be insufficient to increase dramatically the probability of

eliminating disease. Our results do not prove that chemotherapy has no benefit but suggest that large numbers of patients will be required in each disease and stage category if randomized trials are to have any chance of detecting a difference between patients who do and do not receive chemotherapy.

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Phase II Study of Mitolactol and Semustine Combination Chemotherapy for Advanced Malignant Melanoma[1]

Brief Reports

E. T. Creagan,* D. L. Ahmann, A. J. Schutt, and S. J. Green[2]

SUMMARY

Twenty-six patients with disseminated malignant melanoma received an oral regimen of mitolactol and semustine (methyl-CCNU). Sixteen patients had received prior systemic therapy, and 16 had visceral involvement. There were no objective regressions among ten patients receiving the drugs as initial systemic therapy. However, one of 16 patients having prior systemic treatment did have an objective response. Hematologic and gastrointestinal toxic effects were well-tolerated.

[Cancer Treat Rep 66:1425–1426, 1982]

Clinically meaningful chemotherapy for patients with disseminated malignant melanoma remains an elusive goal. Dacarbazine (DTIC), traditionally one of the more effective single agents, has achieved a response rate of only 14% (1). Multiagent regimens utilizing DTIC offer no consistent advantage over DTIC alone (2). The nitrosoureas have similarly displayed some antitumor activity against melanoma. In the absence of comparative clinical trials, the efficacy of carmustine (BCNU), lomustine (CCNU), or semustine (meCCNU) seems to be generally equivalent. MeCCNU is somewhat more attractive because of a 26% response rate among patients with primarily visceral-dominant disease (3) and a conveniently administered oral dose every 6 weeks.

Of considerable interest is a report which showed a 25% response rate from a daily oral dose of mitolactol (DBD), a halogenated hexitol, in patients with advanced malignant melanoma (4). The study is particularly noteworthy since responding patients had been previously treated with DTIC and a nitrosourea, a group notoriously resistant to subsequent chemotherapeutic efforts.

DBD plus meCCNU seemed to be an intriguing regimen for patients with advanced malignant melanoma. Each agent penetrates the central nervous system, a significant site for melanoma metastases, and clearly has activity in selected patients with disseminated disease. Experimental studies have shown striking therapeutic synergism in animal tumor models from the combination of dianhydrogalactitol (a related hexitol) and a nitrosourea (5). In addition, there is a reported precedent of enhanced therapeutic activity in melanoma by combining marginally effective agents with unusual mechanisms of antitumor activity (6). Halogenated hexitols are predominantly inhibitors of RNA metabolism (7), whereas nitrosoureas compromise purine nucleotide formation leading to inhibition of DNA synthesis.

We conducted a pilot study of the DBD and meCCNU combination among 11 patients with a spectrum of advanced solid tumors. Toxicity was primarily hematologic, with leukopenia and/or thrombocytopenia affecting six of 11 patients (table 1). Nausea and vomiting were transient and mild. A clinically acceptable dose was determined to be an oral regimen of 60 mg/m²/day of DBD and 135 mg/m²/day of meCCNU every 6 weeks.

MATERIALS AND METHODS

Twenty-six patients with biopsy-proven disseminated melanoma participated in the study. There were 11 males and 15 females, with a median age of 53.5 years. Fourteen patients had received prior chemotherapy, two had received prior hormone therapy, and ten had had no prior systemic therapy. Twenty-two patients had an Eastern Cooperative Oncology Group performance status of 0–1, and four had a status of 2–3; 16 patients had visceral-dominant disease, and ten had soft tissue or nodal involvement.

Response was defined as a $\geq$ 50% decrease in the product of the longest perpendicular diameters of

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TABLE 1.—Hematologic toxicity of DBD + meCCNU chemotherapy in a pilot study of 11 patients with advanced solid tumors

Dose (mg/m²)		No. of patients	No. of courses	Nadir (cells × 10^3/mm³)					
				Wbc count*			Platelet count†		
DBD	MeCCNU			Mean	Median	Range	Mean	Median	Range
40	90	5	6	7.7	6.5	2.3–15.7	312	337	42–822
40	135	3	4	8.0	9.2	3.4–13.3	264	251	23–468
60	135	3	3	8.4	7.3	3.6–18.0	269	114	65–692

*Normal wbc count: > 4.1 × 10^3/mm³.
†Normal platelet count: > 130 × 10^3/mm³.

the most clearly measurable indicator lesion without the development of new lesions or significant symptomatic deterioration. Disease progression was defined as a > 25% increase in the product of any lesion, the appearance of new lesions, or observation of progression by a local physician (for the one patient who did not return).

RESULTS AND DISCUSSION

There were no objective regressions among the ten patients who received DBD and meCCNU as initial treatment (table 2), whereas one of the 16 patients receiving the regimen as secondary systemic treatment had a regression. This patient, a 77-year-old woman, had been treated with PALA and later AMSA; she had a decrease in the size of soft tissue lesions for 6+ months. For all patients, the median survival and time to progression were 4.8 and 1.4 months, respectively.

Mild to moderate nausea, vomiting, or both occurred in 35% of patients. Thirty-eight percent of patients had leukopenia (< 4.1 × 10^3 wbcs/mm³), thrombocytopenia (< 130 × 10^3 platelets/mm³), or both. There were no clinically significant episodes of hemorrhage or infection, and there were no treatment-related deaths.

We were able to administer only approximately 50% of the generally accepted single-agent dose of each agent used in this regimen. In a prior study, which achieved apparent benefit from combination chemotherapy in advanced melanoma, each of the drugs having modest single-agent activity could be given at full dose (6). We recognize that only one third of our patients demonstrated any myelosuppression, and this could have had an impact on our findings. However, it is unclear if higher doses or alternative scheduling would substantially alter our results. Experimental studies show no apparent schedule dependency for a hexitol plus a nitrosourea (5), and the antitumor activity of a nitrosourea may be independent of myelosuppression (8).

In the dose and schedule used in this study, the combination of DBD and meCCNU displayed a small amount of activity against malignant melanoma. A 95% confidence interval for the regression probability is 0.001, 0.20. Surprisingly, the patient who responded was one who had failed two previous cytotoxic trials.

TABLE 2.—Results of treatment, by prior chemotherapy and site of metastasis, in 26 patients with disseminated melanoma*

Treatment	Visceral dominant	Nonvisceral
Primary	0/7	0/3
Secondary	0/9	1/7

*Values = No. of objective regressions/No. of evaluable patients.

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Phase II Study of Methyl-GAG in the Treatment of Esophageal Carcinoma[1,2,3]

Brief Reports

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SUMMARY

Twenty-four patients with epidermoid carcinoma of the esophagus have been treated, in a phase II trial, with methyl-GAG using a weekly schedule. Eighteen patients had received prior chemotherapy with cisplatin-containing combinations. Of 23 evaluable patients, four (17%) had partial remissions, each lasting 2 months. All patients had received prior chemotherapy. Toxic effects were manageable and included mild nausea and vomiting, mucositis, and fatigue. Using this schedule, methyl-GAG had modest activity in esophageal cancer, with acceptable toxicity. Its role in combination chemotherapy remains to be defined.

[Cancer Treat Rep 66:1427–1429, 1982]

Methyl-GAG is a hydrazone derivative which initially entered clinical trial in the mid-1960s. In these early studies, the drug was given as a rapid iv infusion at doses of 3.5–15 mg/kg on a daily treatment schedule. Although substantial antineoplastic activity was noted in patients with acute leukemias, lymphomas, and esophageal carcinoma (in a study by Falkson), toxicity was severe (1–6). Dose-limiting side effects included mucositis, skin ulcerations, diarrhea, and hypoglycemia. Myelosuppression was minimal. Because of its severe toxic effects, interest in the drug waned. Recently, Knight et al (7) restudied methyl-GAG using a weekly schedule. Antineoplastic activity was again seen in a variety of tumors, but toxic effects were markedly decreased. In view of its more acceptable toxicity, a phase II study in esophageal carcinoma, utilizing a weekly schedule, was performed. Preliminary results of this study have been previously reported (8).

MATERIALS AND METHODS

Twenty-four patients with advanced epidermoid carcinoma of the esophagus (the diagnosis having been confirmed by the Department of Pathology, Memorial Sloan-Kettering Cancer Center) were entered in this trial which began in November 1979 and ended in March 1981. All patients had measurable metastatic disease or recurrent tumor that was evaluable by barium esophagram. Methyl-GAG was supplied by the Drug Evaluation Branch of the National Cancer Institute (Bethesda, MD) in 1-g vials. It was reconstituted with 50 ml of normal saline immediately prior to use. The drug was then diluted in 250 ml of normal saline and infused over 1–1.5 hours. The initial dose was 400–500 mg/m² for poor-risk patients (defined as those with a Karnofsky performance status ≤ 60 or having had heavy prior chemotherapy) and 600–700 mg/m² for good-risk patients. Methyl-GAG was given weekly for 4 weeks. After a 1-week break, a second cycle was given, with a 100-mg/m² dose escalation if no significant toxicity had been noted during the first course. If severe toxicity was seen, methyl-GAG was held until the patient had recovered.

Prior to entrance in the study, all patients had a complete history and physical examination, chest roentgenogram, barium esophagram, electrocardiogram, urinalysis, creatinine clearance, and 12-channel biochemical screening profile. A cbc and platelet count and serum creatinine were repeated prior to each treatment. Because hypoglycemia had been noted in earlier studies, a serum glucose level was also measured before each dose.

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Response criteria, including radiologic examples of response as seen by barium esophagram, have been previously reported (9). An adequate trial was defined as at least three doses of methyl-GAG. Informed consent was obtained from all patients prior to entrance in the trial.

RESULTS

Of the 24 patients entered in the study, 23 were evaluable for response and toxicity. One patient was lost to followup after receiving only two doses of the agent. Patient characteristics are shown in table 1. Of note, most of these patients were heavily pretreated, usually with cisplatin and bleomycin or cisplatin, bleomycin, and vindesine combinations (9,10). As was the case with our earlier phase II trials, patients with poor performance status were initially accepted in the study.

Of the 23 evaluable patients, four (17%) had partial remissions, each lasting 2 months. All of these patients had received at least four courses of the cisplatin-containing regimens mentioned above. Three additional patients had minor regressions, which were not of substantial clinical benefit.

Toxicity

Nausea and vomiting were usually quite mild or absent. Alopecia was also uncommon. Mucositis was seen in two patients. Myelosuppression was not seen (median wbc count nadir, 7.6 $\times$ 10^3/mm^3 [range, 3–18.4]; median platelet count nadir, 595 $\times$ 10^3/mm^3 [range, 300–760]).

The dose-limiting toxicity was a syndrome of fatigue and weakness which was severe in four patients. This was usually seen after two to four doses and, if it occurred and the patient had stable disease or response, methyl-GAG was given every 2 weeks. The syndrome was usually ameliorated using this schedule.

Hypoglycemia was not seen.

DISCUSSION

Epidermoid carcinoma of the esophagus remains a difficult disease to treat. Few single-agent chemotherapeutic trials have been performed. Conventional drugs, such as bleomycin, methotrexate, doxorubicin, 5-FU, and lomustine (CCNU), have been studied in small groups of patients and have had minimal or no activity (11–15). Initial studies from Memorial Hospital starting in 1976, involving investigational drugs, demonstrated modest activity for the two-drug combination of high-dose cisplatin and bleomycin infusion, with 19% of patients having complete or partial regressions (9). In patients with metastatic disease, the median duration of response was 6 months. Since this study, two trials of cisplatin alone involving a total of 36 patients have been reported; the overall response rate was 18%, with brief remission durations (16,17).

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total entered	24
Evaluable	23
Median age in yrs (range): 59 (48–80)	
Median performance status (range): 70 (30–80)	
Sex (male:female)	19:5
Prior therapy	
None	1
Chemotherapy	9
Radiation therapy	5
Chemotherapy and radiation therapy	9

A phase II study with the new vinca alkaloid, vindesine, followed our trial with cisplatin and bleomycin. As was the case with the current study involving methyl-GAG, most of these patients were heavily pretreated, usually with the cisplatin and bleomycin combination. An 18% complete and partial response rate was noted, with a 5-month median duration of response (18). Because they have differing mechanisms of action and toxic effects, and appear, at least in some patients, to lack cross-resistance, the three agents (cisplatin, vindesine, and bleomycin) were then combined. This study has now accrued 62 evaluable patients, 19 with metastatic disease and 43 with local-regional disease (10). The complete and partial response rate is 55%; in patients with metastatic disease, the median duration of remission will be approximately 7.5–8 months. Although the more than doubling of the response rate and increased response duration are quite encouraging, it still means that 40%–50% of patients are not responding to our best chemotherapy combination. Thus, the activity of methyl-GAG, although modest, is of interest, especially in view of the prior chemotherapy these patients had received. Whether or not methyl-GAG can be incorporated into a cisplatin-vindesine-bleomycin type combination will depend on the results of a phase I trial, currently in progress.

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Phase II Study of Chlorozotocin in Malignant Melanoma: A Southeastern Cancer Study Group Report[1,2]

Brief Reports

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SUMMARY

Seventy-nine patients with metastatic melanoma received chlorozotocin (150 mg/m²) every 6 weeks. Two complete and five partial responses were observed: 18% in patients who had not previously received chemotherapy and 6% in previously drug-resistant patients. Dose-limiting toxic effects were vomiting and myelosuppression.

[Cancer Treat Rep 66:1431–1433, 1982]

Chlorozotocin is a nitrosourea that was shown in phase I studies (1–3) to be only minimally myelosuppressive at doses of 120–175 mg/m². It had possible activity against malignant melanoma (1,2). In a recently reported phase II study, a response rate of 14% (five responses among 35 patients) was observed in patients with metastatic malignant melanoma (4). To extend that observation, we report here the experience of the Southeastern Cancer Study Group using chlorozotocin in malignant melanoma.

MATERIALS AND METHODS

Seventy-nine patients with incurable malignant melanoma were entered in the study between January 1978 and February 1981. Nine patients were inevaluable because of insufficient data or protocol violation. Of the remaining 70 patients, 47 were previously treated with chemotherapy (excluding nitrosoureas) and 23 were previously untreated. Voluntary informed consent had been given prior to treatment.

Chlorozotocin (150 mg/m²) was given iv every 6 weeks. Complete response (CR) was defined as the complete disappearance of all manifestations of tumor; partial response (PR) was defined as a 50%–100% decrease in the sum of the products of perpendicular diameters of all measured tumors, without the appearance of new tumors; stable disease was defined as less than a PR, with no evidence of disease progression; and disease progression was defined as the appearance of new manifestations of tumor or a > 25% increase in measured tumor area.

Myelotoxicity was defined as follows: grade 1 included a wbc count nadir of 3000–4000/mm³ or a platelet count nadir of 100,000–130,000/mm³; grade 2 included nadirs of 2000–3000 or 50,000–100,000/mm³, respectively; grade 3 included nadirs of 1000–2000 or 25,000–50,000/mm³, respectively; and grade 4 included nadirs < 1000 or < 25,000/mm³, respectively. Dose reductions of 50% were instituted for grade 3 or 4 myelotoxicity. No course was given until the wbc count was > 4000/mm³ and the platelet count was > 100,000/mm³.

RESULTS

The results of the study are summarized in table 1. In the group of 23 previously untreated patients, two patients (9%) had CR lasting 6 and 18 months, and two (9%) had PR lasting 1.5 and 3+ months. Four patients had stable disease for 1+, 1.5, 3+, and 4 months. The total response rate was 18% in previously untreated patients. Nine of the 23 patients had visceral metastases, and four (44%) of the nine had CR or PR.

There were only three patients (6%) with PR and four (9%) with stable disease among 47 patients who had previously received chemotherapy. The durations of PR were 3+, 4+, and 6 months, and the durations of stable disease were 1.0, 1.5, 1.5, and

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TABLE 1.—Response to chlorozotocin

	Total No. of patients	No. of patients (%)				
		CR	PR	Stable disease	Disease progression	Death
Previously untreated	23	2 (9)	2 (9)	4 (18)	13 (56)	2 (9)
Previously treated	47	0	3 (6)	4 (9)	32 (68)	8 (17)

2+ months. Two (18%) of the 11 patients with visceral metastases had PR, and one (3%) of the 36 patients with cutaneous and/or nodal melanoma had PR.

A tabulation of the responding patients is given in table 2. All but one of the seven patients who had CR or PR had visceral metastases in lung or liver. Both patients who had CR had liver metastases. No patient with a low performance status (< 60%) had a PR, but three of the seven responders (including both with CR) had a pretreatment performance status of only 70%.

There were 118 courses of chlorozotocin evaluated for toxicity. Thirteen episodes of grade 2 leukopenia and five episodes of grade 3 leukopenia occurred. One patient developed sepsis during leukopenia. There were 13 episodes of grade 2 and five episodes of grade 3 thrombocytopenia. The lowest platelet count nadir was 10,000/mm^3.

Eight patients received four or more courses of chlorozotocin that were evaluated for toxicity. Three of these patients developed grade 2 thrombocytopenia after the second course and two developed grade 2 leukopenia; two of the episodes occurred in the previously untreated patients. There were six patients with decreases in hemoglobin > 1 g/dl. Two previously treated patients had severe decreases in hemoglobin (nadirs, 6.4–6.7 g/dl) during the first course of therapy.

One patient had drug-related hepatitis and one had drug-related dyspnea, both associated with chlorozotocin administration, which required discontinuation of the drug. Nausea and vomiting were extremely common, although they usually lasted only several hours and were partially responsive to antiemetics. Nine patients had vomiting unresponsive to antiemetics, and one became dehydrated due to intractable vomiting.

DISCUSSION

Chlorozotocin has been shown to be an active drug in disseminated malignant melanoma, with two CRs and a total response rate of 18% in previously untreated patients. This is similar to the 15% response rate reported by Hoth et al (4) in 20 previously untreated patients using chlorozotocin at a dose of 120 mg/m^2. It is significant that CRs were seen in both studies.

These results contrast somewhat with those of Houghton et al (5). In their study of 19 patients, only one response (less than a PR) was observed in 15 patients with prior chemotherapy, and there were no responses in four previously untreated patients (despite use of chlorozotocin at doses of 175–200 mg/m^2). The response rate in previously drug-resistant patients is not different from that which we ob-

TABLE 2.—Characteristics of patients with CR or PR

Accession No.	Prior chemotherapy	Pretreatment performance status	Sites of tumor metastases	Response	Duration of response (mos)
11002	No	70%	Liver, nodes	CR	18
11022	No	70%	Liver, lung, nodes, skin	CR	6
29005	No	80%	Lung, small intestine	PR	3+
29001	No	90%	Liver, nodes	PR	1.5
23001	Yes	70%	Lung, bone, skin	PR	6
28005	Yes	100%	Lung	PR	4+
22002	Yes	90%	Nodes	PR	3+

served (6%). Since only four patients had not received prior drugs, the failure of Houghton et al to observe any responses is not significant and is consistent with our response rate of 18%.

More myelotoxicity was seen in this study, which used a 25% higher dose of chlorozotocin, than was seen in the previous report by Hoth et al (4). However, we observed less myelosuppression than did Houghton et al (5), who used doses of 175–200 mg/m^2. In this series, there was evidence of cumulative myelosuppression in some patients who received four or more courses.

We conclude that although some activity of chlorozotocin has been observed (which is similar to that of other nitrosoureas), the only meaningful role of chlorozotocin in management of malignant melanoma will likely be in combination with other more myelosuppressive chemotherapeutic agents. In such trials of combinations, chlorozotocin cannot be regarded as a nonmyelosuppressive drug. Cautious phase I trials to define the maximally tolerated dose of the combinations containing chlorozotocin will be required to avoid severe or cumulative myelosuppression.

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Changes in Intestinal Permeability to Lactulose Induced by Cytotoxic Chemotherapy[1]

Brief Reports

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SUMMARY

Intestinal function during cytotoxic therapy was studied in nine patients with nonintestinal lymphoma. An early, marked, and rapidly reversible increase in passive permeability, as disclosed by the lactulose test, was observed without concomitant changes in active absorption or in intestinal histology. The possibility of a toxic effect by cytotoxic drugs on intestinal intracellular junctions is discussed.

[Cancer Treat Rep 66:1435–1436, 1982]

The high cellular proliferation rate of the intestinal epithelium makes it particularly vulnerable to the effects of cytotoxic drugs and ionizing radiation (1,2). Structural and functional alterations in the mucosa of the small intestine have been described in animals and in man during treatment with antimetabolites such as aminopterin (3,4), methotrexate (5), and 5-FU (6) and with colchicine (7,8) and cyclophosphamide (9).

We studied intestinal permeability as previously described (10), using Menzies' lactulose test (11,12), in nine patients with nonintestinal malignant lymphoma who were receiving cytotoxic therapy. Five patients with Hodgkin's disease were treated with the MOPP regimen (13), and four patients with non-Hodgkin's lymphoma were treated with the CVP regimen (14).

The lactulose test measures by quantitative paper chromatography (15) the minute fraction of lactulose excreted in urine 5 hours after administration of this sugar by hyperosmolar load (7 g of lactulose plus 40 g of sucrose in 100 ml of water). The test was performed before and then 2, 10, 16, and 30 (MOPP patients only) days after the beginning of cytotoxic therapy. In four patients a xylose test and an intestinal biopsy were done before and 10 days after the beginning of treatment.

The results are summarized in table 1. Cytotoxic therapy caused immediate repercussions on intestinal permeability to lactulose, which was more than doubled in all subjects as early as the second day of therapy. Subsequently, the test showed a gradual return to basal conditions. However, the xylose test and intestinal histology were unaltered.

The permeabilization effect observed did not seem to have been associated with a hyporegenerative mechanism. Epithelial hyporegenerative damage would have resulted in a decrease in active transport of xylose and an increase in passive permeability to lactulose, such as that described in villous atrophy of celiac disease (16), with maximum evidence after 7–10 days of cytotoxic therapy. In fact, cytotoxic drugs can only block cellular mitosis in the regenerating zone of the epithelium at the crypt level, and cell migration from the crypt floor to the apex of the intestinal villous is known to take from 5 to 7 days (17,18). During this period the pre-existing differentiated epithelium continues to maintain normal intestinal function.

The early appearance and prompt resolution of the permeability increase to lactulose is better explained by a transient metabolic disturbance. It has been suggested that the intestinal transfer of lactulose occurs via channels associated with junctional complexes between adjacent enterocytes (19). The absorption of lactulose can be potentiated by ingestion of a hypertonic solution (11). The hypertonicity is postulated to increase permeability by making intracellular tight junctions more leaky (11,16). The cytotoxic combination therapy employed may exert a further temporary weakening effect on the same junctions. A study of single cytotoxic drugs and different combinations of sugars as intestinal permeability markers is in progress and should clarify the mechanisms involved.

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TABLE 1.—Lactulose excretion during cytotoxic therapy

Patient No.	Type of lymphoma	Therapy	Urinary lactulose basal level (mg/5 hrs)	% permeability increase (Day)				Xylose test	Intestinal biopsy
				2	10	16	30		
1	Hodgkin's	MOPP	68	142	47	9	3	Normal	No change
2	Hodgkin's	MOPP	55	94	49	27	12		
3	Hodgkin's	MOPP	70	78	55	0	0		
4	Hodgkin's	MOPP	54	107	74	0	14	Normal	No change
5	Hodgkin's	MOPP	72	106	31	0	0		
6	Non-Hodgkin's	CVP	84	116	0	0		Normal	No change
7	Non-Hodgkin's	CVP	48	110	25	0			
8	Non-Hodgkin's	CVP	70	85	17	0			
9	Non-Hodgkin's	CVP	80	100	0	0		Normal	No change

Our data suggest that as an indicator of altered intestinal function during cytotoxic therapy, the lactulose test could be more sensitive than either intestinal biopsy or tests that measure active intestinal transport.

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Phase II Study of Tamoxifen in Patients With Metastatic Malignant Melanoma[1]

Clinical Trials Summaries

Ragnar Telhaug,* Olbjørn Klepp, and Ole Børmer[2]

Several reports have presented evidence of estrogen receptor activity in tumor tissue from 12% to 46% of patients with malignant melanoma (1). Nesbit et al (2) reported in 1979 that four of 26 (15%) patients with advanced malignant melanoma treated with tamoxifen (TAM) had an objective tumor response. Prompted by this report, in December 1979 we initiated a phase II study of TAM in patients with disseminated malignant melanoma, and we report our results here in order to contribute to the overall estimation of the collective response rate.

Twenty-one patients with measurable, disseminated malignant melanoma were included in the study (table 1). Tumor tissue samples for the determination of cytosol hormone receptor concentrations were obtained from seven of the patients before the start of TAM therapy. The patients received TAM at a daily dose of 40 mg/day orally in two divided doses until clear evidence of tumor progression. Prior to initiating TAM therapy and at least every 4–6 weeks during the study period, patients had a complete clinical evaluation, chest roentgenogram, and routine hematologic and blood chemistry tests.

One of the patients achieved an objective improvement. He had lung metastases that decreased > 50% in size after 2 months of TAM treatment. Disease progression occurred 5 months after the start of TAM therapy. Two patients had no change in their tumor lesions for 4 and 5 months, respectively. In both cases the rate of disease progression was comparatively slow both before and after TAM therapy. All of the other 18 evaluable cases showed disease progression within 2–3 months after the start of TAM therapy. None of the seven tumor tissues examined showed estradiol binding activity. No significant side effects of TAM therapy were experienced by any of the 21 patients.

The rate of complete and partial response to TAM in advanced malignant melanoma has not been impressive (3,4). In our study we found one responder among 21 evaluable patients. But even a 5%–10% response rate could be of some clinical interest for the therapy of malignant melanoma, because the side effects of such therapy are negligible compared to those of cytotoxic drugs. So far, the possible therapeutic effect of TAM in malignant melanoma remains uncertain at best.

TABLE 1.—Summary of characteristics of 21 patients treated with TAM

Characteristic	No. of patients
No. of evaluable patients	21
Median age in yrs (range): 55 (29–80)	
No. of males/females	12/9
Prior chemotherapy	8
Performance status	
80–100	10
50–70	9
< 50	2
Dominant metastatic site	
Cutaneous, subcutaneous, and/or lymph nodes only	14
Lung	5
Liver	1
Bone	1
Results	
Partial response	1 (5 mos)
Stable disease	2 (4 and 5 mos)
Disease progression	18 (within 2–3 mos)

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Combination Chemotherapy With Bleomycin and Hydroxyurea in the Treatment of Advanced Head and Neck Cancer[1]

Clinical Trials Summaries

Mario A. Eisenberger,* John Denefrio, Michael Silverman, and Howard E. Lessner[2]

Bleomycin has been shown to cause kinetic perturbations (1,2) and to produce partial cell synchronization in an in vivo melanoma study (1). A 24-hour bleomycin infusion (3) followed by hydroxyurea has been reported to produce a significant (59%) response rate in patients with head and neck cancer. We report here a study of this combination adjusted for use in an outpatient environment.

METHODS

Twenty-four patients with histologically proven squamous cell carcinoma of the head and neck were treated; all patients had measurable disease. Responses were defined as follows: *complete response*—the complete disappearance of all measurable lesions, with pathologic confirmation; *partial response*—a $\geq$ 50% decrease in the sum of the products of the largest perpendicular diameters of measurable lesions without the appearance of new lesions; and *stable disease*—a $<$ 50% decrease or a $<$ 25% increase in measurable parameters for at least 8 weeks. Time to progression and survival time were measured from Day 1 of therapy.

Bleomycin was given at a dose of 15 units/m^2 iv on Day 1, followed by hydroxyurea on Day 8 through Day 15 at a daily oral dose of 1000 mg/m^2 in divided doses, repeated every 2 weeks. After a total dose of bleomycin (300 units) was reached, hydroxyurea alone was continued at the same dose daily or as tolerated until progression of disease.

RESULTS

Of the 24 patients entering the study, 19 were evaluable. Four patients were lost to followup within 4 weeks of therapy, and one patient with evidence of response was inadvertently started on palliative irradiation to the indicator lesion during chemotherapy. Table 1 summarizes patient characteristics, response rates, time to disease progression, and survival. Initial Karnofsky performance status was > 70% for patients who had partial response or stable disease and 50%–60% for nonresponders.

Toxic effects were mild to moderate, with two

TABLE 1.—Patient characteristics and response*

Characteristic	
Sex	
Male	18
Female	1
Primary sites	
Unknown	3
Larynx	5
Oropharynx	3
Floor of the mouth	1
Posterior tongue	1
Anterior pilar	1
Tonsil	1
Nasopharynx	1
Oral cavity, not specified	3
Extension of disease (response)	
Loco-regional	16 (9 PR, 3 SD)
Distant metastatic (below the clavicle)	3 (2 SD)
Response†	
PR	9 (48%)
SD	5 (31%)
Disease progression	5 (31%)
Median time to progression (days)	
PR	130
SD	73
Disease progression	42
Median survival (days)	
PR	250
SD	142
Disease progression	112

*Unless specified otherwise, values = No. of patients. PR = partial response; SD = stable disease.

†Response rates on the basis of all 24 patients entered in the study are: PR, 38% (9/24); SD, 20% (5/24); and disease progression, 20% (5/24).

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patients developing wbc count nadirs of $< 1000/mm^3$ and 12 developing nadirs between 1000 and $3000/mm^3$. Platelet count ranged from 50,000 to $100,000/mm^3$ in three patients. Radiologic evidence consistent with pulmonary fibrosis was noted in one patient (total dose of bleomycin, 300 units). Fever and chills were seen in 16 of 19 patients after bleomycin, and one patient had hydroxyurea-induced nausea and vomiting.

DISCUSSION

Bleomycin used alone on a weekly or twice-weekly schedule produces responses similar to those seen in our series (4), whereas hydroxyurea alone seems to have only marginal activity (4). Although our results do not allow us to make any conclusions about the synergism of this combination, the less intensive schedule in this pilot study produced responses in a significant number of patients. It appears at least as effective as more intensive and/or toxic regimens in the palliation of patients with advanced squamous cell carcinomas of the head and neck.

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High-Dose Medroxyprogesterone Acetate for the Treatment of Advanced Ovarian Carcinoma[1]

Clinical Trials Summaries

Claes Tropé,* John-Erik Johnsson, Kristján Sigurdsson, and Ernst Simonsen[2]

High-dose medroxyprogesterone acetate (MPA) is of significant value in palliative treatment of hormone-dependent neoplasms such as breast (1) and endometrial (2) carcinoma. Progestogens of various kinds have been reported to have activity in patients with ovarian adenocarcinoma, but the results have been conflicting, and the response rates have varied from 5% to 65% (3,4). The aim of this study was to further elucidate the effect of high-dose MPA in ovarian cancer resistant to cytostatic drugs.

Twenty-eight patients with recurrent, advanced (International Federation of Gynecology and Obstetrics stages III and IV), chemotherapy-resistant ovarian carcinoma, with measurable or evaluable lesions, were included in the study. Patient characteristics, response, survival, and toxic effects are shown in table 1.

MPA, a synthetic steroid derived from progesterone (Farmitalia Carlo Erba), was given im in a daily dose of 500 mg for 3 weeks, followed by 500 mg once a week for at least 2 months. Patients with an objective response or stable disease continued treatment with 500 mg every week until disease progression. The results of treatment were defined according to the World Health Organization criteria.

Three patients were not eligible due to early death from tumor. Twenty-five patients with a median age of 61 (range, 43–81) were evaluable; seven patients had stage III and 18 had stage IV disease.

Of the 25 evaluable patients, one achieved partial response (duration, 3+; survival, 4+ months); nine had stable disease (median duration, 5.5+ months; median survival, 6.5+ months); and 15 had progressive disease (median survival, 3 months). The objective response occurred in a 73-year-old woman with undifferentiated carcinoma, stage IV disease. This patient had a > 50% reduction of central pelvic mass and inguinal nodes. Nine (36%) of the 25 patients increased their Karnofsky index by a mean of 20 points.

During drug therapy, there were no changes in blood counts or serum levels of creatinine, bilirubin, alkaline phosphatase, and fasting blood sugar. One patient gained weight due to fluid retention and she also developed high blood pressure. More severe complications such as deep vein thrombosis were seen in two patients, but both had large pelvic tumors and had perhaps developed thrombosis.

The overall response rate was 4%, which is about the same result achieved by Malkasian et al (3) but far from the high response rate of 60% reported by Ward (4). Since most of the epithelial ovarian carcinoma cell types were included in this study, it is reasonable to conclude that objective responses may be obtained only in a minority of patients with ovarian carcinoma resistant to cytostatic drugs. However, we have used MPA as a third-line therapy and when chemotherapy is used as a second- or third-line therapy, the overall response rate is also very low (5).

The present investigation shows the MPA in patients with ovarian cancer previously treated with chemotherapy is not an effective treatment.

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TABLE 1.—Pretreatment features and response to MPA therapy*

Patient No.	Age	Histology	Surgery	Previous therapy		Clinical stage	Response	Duration (mos)		Pretreatment-posttreatment Karnofsky index	Side effects
				Irradiation	Chemotherapy			Remission	Survival		
1	73	AC	SOEB	+	Melf	IV	PR	3+	4+	80–100	None
2	45	SMD	HIT + SOEB	+	Melf + Adr	IV	SD	6+	7+	80–100	H + WG
3	63	SMD	Expl lap	+	Melf + Adr	IV	SD	6+	7+	80–100	None
4	67	SMD	SOEB	+	Melf	III	SD	6+	7+	80–80	None
5	69	AC	Expl lap	+	Melf	III	SD	7+	8+	100–100	T
6	68	SMD	Expl lap	+	Melf	III	SD	5+	6+	80–80	None
7	58	SPD	Expl lap	+	Melf	III	SD	5+	6+	80–100	None
8	74	SHD	SOEB	–	Melf + Adr	III	SD	4+	5+	80–100	None
9	69	SHD	HIT + SOEB	–	Melf + Adr	IV	SD	3+	4+	80–100	None
10	76	AC	Expl lap	+	Melf + Adr	IV	SD	3+	4+	80–100	None
11	43	EHD	SOEB	–	Melf + Adr + Cis	IV	PD		6	60–60	None
12	61	AC	FB	–	VM-26	IV	PD		6+	80–100	None
13	63	AC	Expl lap	–	Melf + Adr	IV	PD		6+	80–100	None
14	73	AC	FB	–	VM-26	IV	PD		5	80–80	T
15	61	AC	HIT + SOEB	+	Melf	IV	PD		3	80–80	None
16	61	AC	Expl lap	–	Melf	III	PD		3	50–50	None
17	70	AC	Expl lap	–	Melf + Adr + Cis	IV	PD		3	80–80	None
18	59	SPD	SOEB	–	Melf + Adr	IV	PD		3	80–80	None
19	81	AC	SOEB	+	Melf	III	PD		4+	80–80	None
20	55	SHD	SOEB	–	Melf + Adr	IV	PD		3	80–80	None
21	50	SPD	HIT + SOEB	+	Melf + Adr + Cis	IV	PD		3	60–60	None
22	50	SPD	HIT + SOEB	–	Melf + 4-epi	IV	PD		3	60–60	None
23	50	SPD	SOEB	+	Melf	IV	PD		3	60–60	None
24	49	EHD	HIT + SOEB	–	Melf + Adr	IV	PD		3	60–60	None
25	43	SPD	SOEB	–	Melf + Adr	IV	PD		3	80–80	None

*AC = anaplastic carcinoma, SMD = serous moderately differentiated, SPD = serous poorly differentiated, SHD = serous highly differentiated, EHD = endometrioid highly differentiated, SOEB = salpingo-oophorectomy, HIT + SOEB = total hysterectomy + salpingo-oophorectomy, Expl lap = explorative laparotomy, FB = fine needle biopsy, Melf = melphalan, Adr = doxorubicin, Cis = cisplatin, 4-epi = 4′-epi-doxorubicin, VM-26 = teniposide, PR = partial response, SD = stable disease, PD = progressive disease, H = hypertension, WG = weight gain, and T = deep thrombosis.

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Trial of Tamoxifen at a Dose of 40 mg Daily After Disease Progression During Tamoxifen Therapy at a Dose of 20 mg Daily[1]

Clinical Trials Summaries

J. F. Stewart,* M. J. Minton, and R. D. Rubens[2,3]

Tamoxifen, an antiestrogen, is an effective treatment in a number of postmenopausal, and probably premenopausal, women with advanced breast cancer (1). Although it has been suggested that higher response rates are seen in patients treated with 40 mg daily rather than 20 mg daily (2) and although there has been a case report of further disease remission with 20 mg twice daily after disease progression during therapy with 10 mg twice daily (3), the optimum dosage has not been established.

We have conducted a prospective nonrandomized trial to assess the response of advanced breast cancer to 20 mg of tamoxifen twice daily after disease progression during therapy with 10 mg twice daily.

The patients studied had histologically confirmed, locally advanced inoperable or metastatic breast cancer that was progressing during treatment with 10 mg of tamoxifen twice daily with or without 5 mg of prednisolone twice daily. After physical examination (with measurement of all palpable lesions and photography of visible lesions), chest radiograph, cbc, biochemical screen (including liver function tests), and isotopic bone scan (with radiographs of areas of increased uptake), the dose of tamoxifen was increased to 20 mg twice daily. Response was assessed by International Union Against Cancer criteria (4). Estrogen receptor (ER) analysis had previously been determined by the method of King et al (5). Patients with rapidly progressing visceral disease after the lower dose of tamoxifen were treated directly with chemotherapy and excluded from this trial.

Thirty-six consecutive patients were entered in this study; 30 were evaluable. Six patients were inevaluable for the following reasons: no assessable lesions (one patient), failure to take treatment because of side effects (three), loss to followup (one), and early death (one). Characteristics of the 30 evaluable patients are shown in table 1.

No objective responses to the higher dose of tamoxifen were observed. Seven patients had stable disease for 2–15 months, two who had responded to previous endocrine therapy (tamoxifen, prednisolone) and five who had had no prior response. These patients comprised two with ER+ ($\geq$ 5 fmols/mg of cytosol protein) tumors, one with an ER– ($<$ 5 fmols/mg of cytosol protein) tumor, and four with unknown tumor ER status. The other 23 patients all had progressive disease. Median time to treatment failure was 2 months (range, 1–15), and median survival from study entry was 11 months (range, 2–17).

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total	30
Mean age at entry in study, in yrs: 64.9	
Stage at presentation	
I or II	15
III or IV	15
Median disease-free interval, in mos (range): 20 (0–204)	
ER status*	
+	16
–	2
Not known	12
Previous systemic therapy for advanced disease	
Tamoxifen	25 (9)†
Tamoxifen + prednisolone	5 (5)
Estrogens	2 (1)
Androgens	2 (1)
Prednisolone	4 (2)
Progestogens	1 (1)
Chemotherapy	7 (3)
Sites of disease	
Soft tissue	26
Bone	5
Viscera	7

*+ = $\geq$ 5 fmols/mg of cytosol protein; – = $<$ 5 fmols/mg of cytosol protein.

†Values in parentheses = No. of responses.

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[2]Imperial Cancer Research Fund, Breast Cancer Unit, Guy's Hospital, London, England.

[3]We thank R. J. B. King for performing the estrogen receptor analysis.

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These results indicate that escalation of tamoxifen dosage to 20 mg twice daily after disease progression during therapy with 10 mg twice daily does not lead to further response and that alternative therapy, either endocrine or cytotoxic, should be given.

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Phase II Trial of Tamoxifen in Malignant Melanoma[1,2]

Cynthia G. Leichman, Michael K. Samson,* and Laurence H. Baker[3]

Clinical Trials Summaries

In 1976, Fisher et al (1) initiated a study testing for the presence of hormone receptors in melanoma biopsy specimens. The underlying premise that melanoma might be a hormonally sensitive tumor was based on observations that the natural history of the disease differed in men and women. Subsequent to the demonstration of cytoplasmic estrogen receptors in melanoma, several studies were initiated using hormonal manipulation in the treatment of disseminated malignant melanoma. Early studies suggested that regression of disease could be achieved with estrogen compounds (2).

Following the results of treatment of hormonally sensitive breast cancer with antiestrogens, similar therapy was attempted in disseminated melanoma. Several studies have produced mixed results ranging from no response to stabilization of disease to brief partial responses in small numbers of patients to a sporadic complete response (3–5). Increasing the doses of tamoxifen to obtain higher initial blood levels was no more successful in producing response. These studies revealed that the presence or absence of hormone receptors had no relation to clinical response or median survival (3). Performance status at the initiation of therapy was the most significant predictor of survival.

Between 1978 and 1980, the Wayne State University Department of Oncology conducted a phase II clinical trial of tamoxifen in disseminated malignant melanoma. The standard dose of tamoxifen, 10 mg orally twice a day, was used.

Twenty patients ranging in age from 34 to 81 years (median, 56) were entered in the study. Nine were female, 11 male. All but two patients had received previous chemotherapy or immunotherapy; most had been treated with multiple drugs, either dacarbazine alone or in combination. Disease-free interval ranged from zero (disseminated at diagnosis) to 8 years (median, 25 months). Median Karnofsky performance status at the time of entry into the protocol was 80 (range, 40–100). Two patients were unevaluable because of early death, defined as death within 2 weeks of entry into the study.

Of the 20 patients studied, 16 had predominantly soft tissue disease, three had lung disease, and one had lung and soft tissue disease.

Estrogen receptor protein was assayed in 12 of the 20 patients; three were not biopsied because the disease was primarily in the lung, one refused biopsy, and four had no readily assessable tumor. The results from one of the tumors assayed were invalid because of radioactivity in the sample. Of the 11 remaining assays, nine were negative for estrogen receptor protein and two were reported as "low": 3.6 and 4.0 fmols/10 mg wet weight of tissue.

Eighteen patients were evaluable for response (two were unevaluable because of early death). One patient had a partial response after 4 weeks of treatment, with regression of skin nodules. This response lasted for 3 weeks before the appearance of new disease. The estrogen receptor assay was negative in this patient. Toxic reactions were rare and consisted of mild nausea, weight gain, and hot flashes.

From the results of this study, we conclude that tamoxifen is not an effective agent in the treatment of disseminated malignant melanoma in patients who have received prior chemotherapy.

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[2]Supported by contract N01-CM-07404 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health and Human Services.
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Combination Antiemetics

Nausea and vomiting secondary to agents such as cisplatin are a major cause of morbidity in cancer chemotherapy (1). Gastrointestinal upset occurs in as many as 83% of these patients and may, in fact, be the limiting factor in chemotherapy (2). At the present time, only three major drug classes have been considered to be effective antiemetics: antihistamines, anticholinergics, and dopamine antagonists. These drugs are thought to act on the chemoreceptor trigger zone and/or emetic center in the medulla oblongata (2) where recent neuropharmacologic techniques have demonstrated the presence of histamine H_1 (3), muscarinic cholinergic (4), and dopamine (5) receptors. However, no single agent has yet proved to be effective in relieving severe nausea and vomiting. It thus follows logically that antiemetic efficacy might be improved by combining drug effects at all three proposed sites of action.

Morran et al (1) reported that the combination of fluphenazine and nortriptyline significantly reduced the incidence and severity of nausea and vomiting during cancer chemotherapy. The effectiveness of nortriptyline, an antidepressant, was thought to be related "to the relief of psychiatric morbidity which is common in patients receiving chemotherapy" (1). More likely, nortriptyline's antiemetic actions derive from its potent antihistamine and anticholinergic properties which have been documented in neurotransmitter receptor binding studies (6). In combination with fluphenazine, simultaneous blockade of histamine H_1, muscarinic cholinergic, and dopamine receptors occurs.

Other evidence also supports this hypothesis. Eight oncology patients at Stanford University Hospital were treated with prochlorperazine (10 mg iv every 4 hours) and diphenhydramine (25 mg iv every 4 hours) beginning 30 minutes prior to a 24-hour cisplatin infusion. Each patient had previously received between two and 11 cycles of cisplatin. Seven of the eight patients reported subjective preferences for the drug combination over any single agent used during prior chemotherapy cycles. The eighth patient denied any significant decrease in the amount of nausea and vomiting experienced during the cisplatin infusion. As with fluphenazine and nortriptyline, the simultaneous use of prochlorperazine and diphenhydramine results in blockade of dopamine, muscarinic cholinergic, and histamine H_1 receptors.

In summary, dopamine, cholinergic, and histamine antagonists all display antiemetic actions which are mediated via different pathways (2). Current neuropharmacologic techniques such as receptor binding assays provide a rapid and accurate measure of antiemetic effects at central nervous system receptor sites. It seems logical to employ these techniques to maximize antiemetic actions in the central emetic pathway. We are presently conducting a clinical trial to further investigate this hypothesis.

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Streptozocin-Induced Eosinophilia and Fever: A Case Report[1]

Streptozocin, a nitrosourea compound, has been used alone or with 5-FU to treat pancreatic islet cell carcinoma and carcinoid tumors (1–4). Its major toxic effects are renal and gastrointestinal. Uremia

[1]Supported by a grant from the General Clinical Research Centers Branch (RR-53).

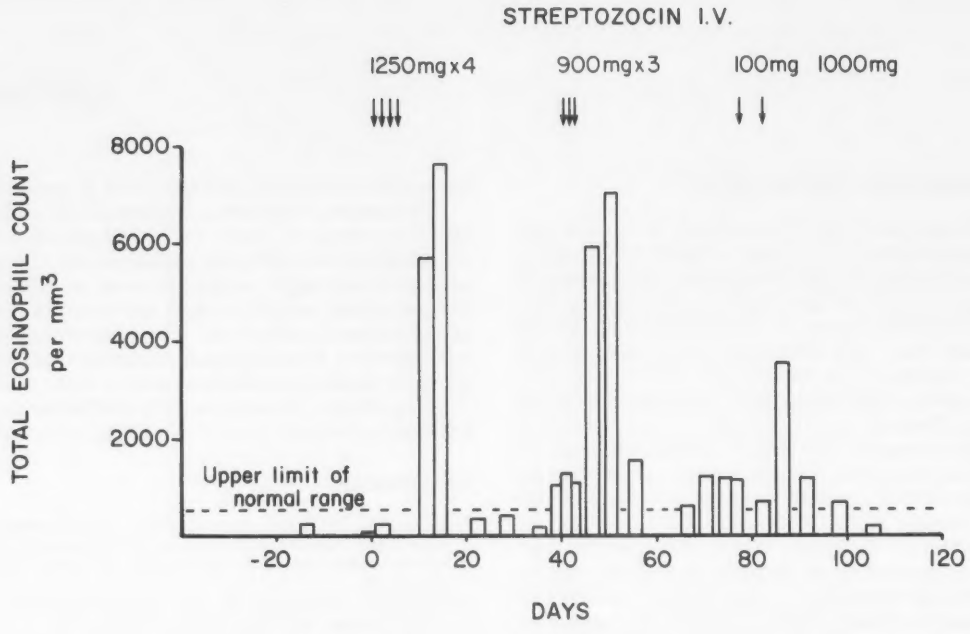

FIGURE 1.—Streptozocin-induced eosinophilia.

has occurred in a significant number of patients, and severe nausea and vomiting are common. Myelotoxicity occurs occasionally and hepatotoxicity has been reported in a few patients (1–4).

We are reporting the present case because of the relationship between streptozocin therapy and the development of fever, eosinophilia, and jaundice. Challenge with a lower dose of streptozocin reproduced fever and eosinophilia but not jaundice.

CASE REPORT

A 51-year-old man was admitted to the Clinical Research Center of Montefiore Hospital and Medical Center for the treatment of metastatic trabecular carcinoma of the skin. This tumor is believed to be a variant of carcinoid tumors arising in the skin (5). The primary malignancy was excised from the right buttock in June 1979 and regional node involvement was documented in April 1980. After initial radiation therapy, streptozocin and 5-FU therapy was instituted on July 9, 1979. The dose was 1250 mg/day of streptozocin and 900 mg/day of 5-FU for 4 days (fig 1). Prochlorperazine was administered to control vomiting. Ten days after completion of therapy, jaundice and marked peripheral blood eosinophilia were noted. The highest bilirubin was 3.9 mg/dl.

A second course of chemotherapy was instituted on August 18, 1979. The streptozocin dose was 900 mg and the 5-FU dose was 625 mg. This cycle was aborted after three doses because of fever, eosinophilia, and a bilirubin of 3.36 mg/dl. Rechallenge with a single dose of 100 mg of 5-FU followed 3 days later by 1000 mg did not produce side effects. Rechallenge with 100 mg of streptozocin was also uneventful, but a 1000-mg iv dose was followed 1 day later by a rectal temperature of 101.8°F and 3 days later by marked eosinophilia. Liver function abnormalities were not seen.

DISCUSSION

In our patient, hepatotoxicity and eosinophilia were noted 10 days after the first course of chemotherapy. In view of the multiplicity of drugs being used (5-FU, streptozocin, and prochlorperazine), it was not possible at first to implicate streptozocin as the causative agent. During the second course of therapy, the same side effects developed after only three doses of streptozocin and 5-FU without prochlorperazine. Although only fever and eosinophilia were

reproduced after a single 1000-mg challenge dose of streptozocin, it is probable that jaundice would have recurred with the higher doses that were given in the first and second courses.

Hepatotoxicity is a known side effect of streptozocin therapy, although most investigators have found it difficult to separate liver function test abnormalities due to the disease from those due to the drug (1–4). Jaundice is rarely seen.

It appears that transient hepatic enzyme elevations occur frequently with streptozocin therapy, but that ours is the first reported patient who developed transient fever, eosinophilia, and bilirubinemia clearly related to streptozocin therapy. This combination of side effects strongly suggests an allergic reaction to streptozocin.

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ANNOUNCEMENTS

Update: Varicella Zoster Immune Globulin

Case by case review, which has been required for release of varicella zoster immune globulin (VZIG), is being suspended because distribution experience has shown that supply of this newly licensed product is adequate for all indicated uses. Initially there was little basis for projecting the potential demand following licensure; to insure that the supply would be adequate to meet the most critical requirements, the case by case review procedure was established. It is now evident that supply considerations will not require this review although any physician who is unfamiliar with the product indications or dosage is encouraged to seek the advice of staff physicians at the local distribution center.

VZIG produced by the Massachusetts Public Health Biologics Laboratories (MPHBL) and distributed by the American Red Cross Blood Services-Northeast Region (ARCBS-NR) is licensed by both the US and Canadian Bureaus of Biologics. It is made from plasma obtained by ARCBS-NR from healthy volunteer donors and is intended for passive immunization of susceptible immunodeficient individuals following exposure to chickenpox or zoster. Indications for use in those susceptible include congenital or acquired immunodeficiency, leukemia, lymphoma, or immunosuppressive therapy, combined with significant exposure to varicella or zoster patients. Use is also recommended for newborns whose mothers have onset of chickenpox less than 5 days before or 48 hours after delivery and for bone marrow transplant recipients regardless of previous varicella infection.

During the first year under FDA licensure, 6258 vials were distributed through regional depots established by ARCBS-NR in the US and Canada, 475 vials were distributed through MPHBL for use within the state of Massachusetts, and 297 vials were shipped overseas. Cooperative efforts between ARCBS-NR and MPHBL to identify and recruit normal volunteer donors with stable high-titer antibodies to varicella zoster virus have increased efficiency of production by lessening dependence on screening of random blood donations. The high demand months of winter and spring are upon us, and we look forward to being able to supply VZIG wherever the need arises.

For more information, contact: The American Red Cross Blood Services-Northeast Region (617) 449-0773.

Ions, Cell Proliferation, and Cancer

Lake Placid, New York, July 14–16, 1982

This symposium, sponsored by the National Cancer Institute and the W. Alton Jones Cell Science Center, will be held at the W. Alton Jones Cell Science Center, Lake Placid, New York. The focus will be on the roles of sodium, potassium, calcium, magnesium, pH, cyclic nucleotides, and calcium-binding proteins in a wide variety of proliferating systems and in cancer development. Techniques and methodology will also be discussed. The symposium co-chairmen are A. L. Boynton, W. L. McKeehan, and J. F. Whitfield.

For more information, contact: Dr. W. L. McKeehan, W. Alton Jones Cell Science Center, Old Barn Rd, Lake Placid, NY 12946.

Urologic Cancer Course

Boston, Massachusetts, September 22–24, 1982

This intensive 3-day course, to be held at the Parker House Hotel, will provide an overview of the clinical and pathophysiologic basis for the diagnosis and treatment of patients with urologic cancer. The course will emphasize the multidisciplinary approach that constitutes the foundation of contemporary care for patients with urologic cancers. Principles derived from the disciplines of urologic surgery, medical oncology, and radiation therapy will be highlighted and incorporated into a comprehensive consideration of therapeutic practices. Although teaching will be primarily provided through lectures, panel

discussions addressing major current controversies in urologic cancer will be an integral portion of the course. A comprehensive syllabus will be provided.

Guest faculty will include Drs. Lawrence H. Einhorn, Donald Skinner, Willet F. Whitmore, Jr, and Leonard Zinman. The Harvard faculty participants will include leaders in urologic cancer from the Sidney Farber Cancer Institute, Brigham & Women's Hospital, Joint Center for Radiation Therapy, Children's Hospital Medical Center, Beth Israel Hospital, and Massachusetts General Hospital.

The course fee is $325.00.

For further information, contact: Harvard Medical School, Department of Continuing Education, Boston, MA 02115 (617-732-1525).

Fifth Annual Bristol-Myers Symposium on Cancer Research—Chromosomes and Cancer: From Molecules to Man

Chicago, Illinois, October 18–19, 1982

This symposium, sponsored by Bristol-Myers Company and to be held at the University of Chicago, will focus on the consistent chromosome abnormalities that occur in human tumors and will explore the use of new techniques that can elucidate the role of chromosome abnormalities in malignant transformation. Over 20 lectures will cover the following topics: chromosome structure, gene organization and control of gene expression; nonrandom chromosome aberrations in human leukemia and embryonic tumors; and the application of new techniques to determine the effect of chromosome abnormalities on gene function, including prophasing chromosomes, chromosome sorting, somatic cell hybrids, and in situ hybridization.

Speakers will include June Biedler, Anthony Carrano, Pierre Chambon, Geoffrey Cooper, Carlo Croce, H. John Evans, Uta Francke, Harvey Golomb, Ann Henderson, Ulrich Laemmli, Eric Lester, Felix Mitelman, Janet Rowley, Frank Ruddle, James Shapiro, Michael Sheffery, Ellen Soloman, Jack Spira, Hewson Swift, John Ultmann, David Ward, Harold Weintraub, Bryan Young, and Jorge Yunis.

For further information regarding registration, contact: Dr. Janet D. Rowley, or Dr. John E. Ultmann, The University of Chicago Cancer Research Center, Box 444, 950 E 59th St, Chicago, IL 60637.

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